Biodegradable polylactic acid cellulose nanocomposites

Fredric Mosley

Degree Thesis
Materials Processing Technology
2018

EXAMENSARBETE	
Arcada	
Utbildningsprogram:	Process- och Materialteknik
Identifikationsnummer:	
Författare:	Fredric Mosley
Arbetets namn:	Bionedbrytbara polylaktid cellulosa nanokompositer
Handledare (Arcada):	Stewart Makkonen-Craig
Uppdragsgivare:	

Sammandrag:

Syftet med detta slutarbete var att studera nanocellulosa och användning av nanocellulosafibriller som fyllnadsmedel och förstärkning i den bionedbrytbara plasten PLA. Biopolymeren nanocellulosa kan användas i en stor mängd produkter för att bland annat ersätta oljebaserade plaster och minska anhopningen av icke-bionedbrytbart avfall. Nanocellulosafibriller (CNF) kan isoleras ifrån växter med nedbrytande metoder och därefter behandlas i en vattensuspension för att minimera mängden aggregat orsakade av reaktiva hydroxylgrupper. Genom att lösa upp PLA i kloroform samt överföra cellulosananofibrillerna till samma lösningsmedel kunde båda komponenterna blandas och gjutas för att uppnå bästa möjliga partikelfördelning. I ett försök att förbättra hopkopplingen mellan de två komponenterna modifierades nanocellulosafibrillernas ytkemi genom esterifiering av polära hydroxylgrupper med hydrofobiska acetylgrupper. De producerade filmerna med 2 respektive 5 viktprocent nanocellulosafibriller påvisade högre brottgräns än film av ren PLA producerad med samma metod. Undantagsvis hade den acetylerade filmen med låg mängd CNF lägre brottgräns. Svepelektronmikroskopi påvisade att de acetylerade fibrillerna hade en tydligt bättre vätbarhet och kunde användas i mindre krävande sammansättningsmetoder.

Nyckelord:	Nanocellulosa CNF polylaktid PLA komposita material
Sidantal:	
Språk:	Engelska
Datum för godkännande:	

DEGREE THESIS	
Arcada	
Degree Programme:	Materials Processing Technology
Identification number:	
Author:	Fredric Mosley
Title:	Biodegradable polylactic acid cellulose nanocomposites
Supervisor (Arcada):	Stewart Makkonen-Craig
Commissioned by:	

Abstract:

This thesis focused on gathering information and reviewing nanocellulose and cellulose nanocomposites. Nanocellulose is a biopolymer that could be used in a wide range of applications to minimize oil dependency and accumulation of non-degradable waste. Nanocellulose can be isolated from plants and processes often have to be repeated many times to reach nanoscale dimensions. Its affinity for water limits drying which means that aqueous suspensions have to be taken into account in composite production. Cellulose nanocomposites can be produced using conventional compounding methods, but mismatching material properties will negate the desired effects as particles are not able to form a network throughout the composite material. A common method for creating nanocomposites is solvent casting which uses solvents to dissolve the matrix material allowing particles to be dispersed. In the experimental work polylactic acid (PLA) films with small amounts (2 and 5 wt%) of cellulose nanofibrils (CNF) were produced. An acetylation treatment of CNF was done to make the surface hydrophobic for better compatibility with the PLA matrix and films with both acetylated and unmodified CNF were produced. Films were visually analyzed, then tensile tested. The ultimate tensile strength of films with unmodified and modified CNF were compared to neat PLA showing that the ultimate tensile strength increased with CNF content. Exceptionally 2 wt% acetylated CNF/PLA exhibiting lower strength than that of neat PLA. SEM imaging of the films showed the existence of interconnected fibril networks in all films and that acetylated CNF had been wetted by the polymer whereas unmodified CNF had formed a network with minimal interfacial adhesion to the matrix.

Keywords:	Nanocellulose CNF cellulose nanofibrils polylactic acid PLA composites
Number of pages:	
Language:	English
Date of acceptance:	

CONTENTS

LIST OF ABBREVIATIONS

AGU	anhydroglucose unit
BC	bacterial cellulose
CNC	cellulose nanocrystals
CNF	cellulose nanofibrils
DCM	dichloromethane
DS	degree of substitution
DP	degree of polymerization
PET	polyethylene terephthalate
PLA	polylactic-acid
SEM	scanning electron microscophy
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl

Figures

Tables

FOREWORD

I would like to thank the Department of Energy and Materials Technology at Arcada for maintaining an open and supportive study environment and Heli Kangas from VTT Technical Research Centre of Finland for kindly donating nanocellulose for my project and welcoming me to learn and ask questions.

Lastly, I would like to express my sincere gratitude and appreciation to my supervisor Stewart Makkonen-Craig for his guidance and devotion to teaching which continues to inspire me and other students.

1 INTRODUCTION

1.1 Background

Petrochemical products have caused severe environmental and disposal related problems due to lack of consideration for end-of-life management and accumulation of non-degradable waste can be seen especially in landfills and maritime environments where it interacts with the ecosystem (Ding et al. 2018, Law, 2017). Initiatives to minimize waste and reach environmental sustainability continuously push research and development in the field of derivatives such as green composites (Rissanen, 2016). Polymers of biological origin, often referred to as biopolymers, are seen as a promising alternative for reducing the amount of non-biodegradable waste ending up at landfills. There are two ways to tackle this problem, reducing the impact of conventional production or replacing the precursor with new sustainable alternatives (Zhu et al. 2016). The drive for sustainable materials stems mostly from policy, international agreements for reducing the consumption of fossil fuel and not from particularly favorable performance or economical advantage (Zhu et al. 2016, Swain et al. 2018). As bioderived polymers mostly have properties inferior to conventional materials, functional composites with application specific chemical and mechanical qualities can be considered (Thakur, 2018). The most abundant organic renewable resource is cellulose, which can be found in plants and bacteria. In Finland 86% of land is forest covered (Sutinen, 2017). The annual growth of wood biomass surpasses felling and other losses by 20 million m^3 and according to the Finnish Natural Resources Institute (2017) harvesting approximately 81 million cubic meters of stem wood per year will be sustainable during 2011-2020. The decline in demand for paper products is of concern for countries with a long history in the forest industry however, the existing know-how and equipment form a solid platform for developing value-added products such as nanocellulose to stay economically competitive (RISE, 2015, Sutinen, 2017). It is expected that the gross world product for nanocelluloses will reach $600 billion by 2020 (Endes et al. 2016). Although cellulose has been utilized as a pulp in papermaking since 8 BCE and what can be classified as microfibrillated cellulose later also been used in the industry in filters, the elementary constituents cellulose nanofibrils (CNF) and cellulose nanocustals (CNC) were officially recognized in the late 1970s.

(Isogai et al. 2010). Polylactic acid (PLA) is a compostable biopolymer frequently used in packaging because of its appearance, strength and O_2, CO_2 permeability coefficients close to that of common packaging material PET. It is thermally unstable and has a low glass transition temperature which limit its use.

1.2 Objectives

The main objective of this thesis was to learn about applications, trends and production methods of nanocellulose and cellulose nanocomposites by reading journals and comparing works. The experimental work focused on producing PLA films with a low content of cellulose nanofibrils (CNF) for studying the difficulties of implementing CNF in fully biodegradable "green" composites as well as evaluating the performance of resulting films. The laboratory experiments were to be performed at Arcada's chemistry lab using existing laboratory equipment and mechanical testing of the films in Arcada's plastics lab. SEM images were planned to be taken at the University of Helsinki.
In summary, the objectives were:

- To study the trends, demand and markets for nanocellulose by reading journals and comparing works.
- Produce cellulose nanocomposites and study the interface between components and overall performance.

1.3 Approach

A comprehensive study of recently published literature was conducted comparing the existing works and gathering information relating to the subject. The literature review gives the reader a holistic understanding of current trends and methods and the experimental shows a method for producing CNF/PLA composites. This work is divided into four chapters of which the first lays the foundation by stating the reason for studying this subject, methods for doing so and the main objectives and approach. The second chapter reviews nanocellulose and selected applications. In the third chapter performed experiments and obtained data is presented in chronological order from start to finish. Lastly, in the fourth and final chapter the results are compared and discussed.

2 LITERATURE REVIEW

This chapter explores the nature of cellulose and common structures, specifically cellulose nanofibrils (CNF) and cellulose nanocrystals (CNC). Bacterial cellulose (BC) is included shortly as a frame of reference and to give the reader a holistic view of existing technologies.

2.1 Cellulose

The world's most abundant biopolymer, cellulose, can be found in plants, algae, fungi, minerals and animals (Heinze, 2016). Plant cells have layered extracytoplasmic structures of which the middle layer of the secondary wall contributes to maintaining structure and rigidity (Ng et al. 2015). This layer of lignocellulosic fibers shown in Figure 1 consists of cellulose microfibrils surrounded by a matrix of polysaccharides and glycoproteins, lignin and pectin (Ng et al. 2015). The compact fiber also contains non-structural components like inorganic salts and waxes. The purest source is cotton fibers, containing 95-97% cellulose with a crystallinity around 70%. Birch and pine stems consisting of approximately 40% cellulose also contain a large percentage of lignin and other polysaccharides known as hemicelluloses (Räisänen & Athanassiadis, 2013, Klemm et al. 2015).

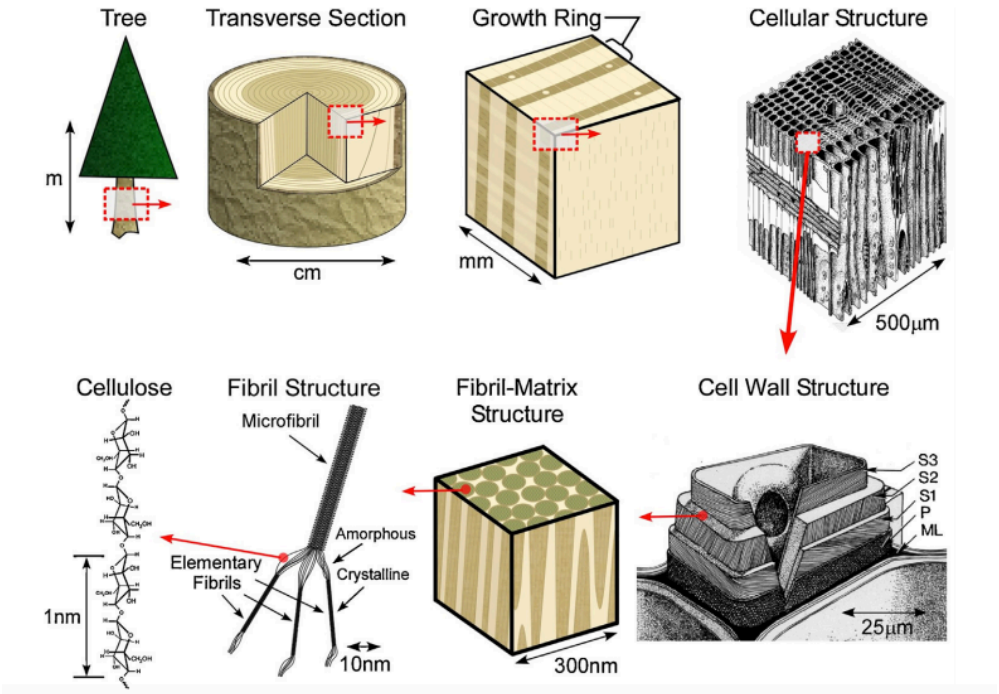


Figure 1: Hierarchical structure of wood (Dufresne, 2013)

The molecular structure of cellulose is independent of source, but the chain length is variable and other factors such as botanical origin, growth conditions, soil, climate circumstances and age affect the overall chemical composition of the unprocessed fibers. About 30 to 100 molecules are linked during natural biosynthesis processes to form the elementary fibrils of cellulose (Ng et al. 2015). It has been proposed that carbohydrates were the first biopolymers formed on earth (Dufresne, 2018). Initially all compounds with the general formula C_xH_2O thought to be "hydrated carbon" until it was discovered that the arrangement of molecules had little to do with water (Dufresne, 2018). The properties of polysaccharides depend on their constituents, configuration and bond type. For example, linking single sugars glucose and fructose together yields common table sugar, sucrose. Disaccharides form in the cell by condensation reactions, where one monomer loses a hydrogen atom and the other monomer a hydroxyl group. They are then linked together by covalent, glycosidic bonds that appear in two instances, alpha and beta. Sugars serve a variety of functions and are most important for storing energy in cells (Dufresne, 2018). Storage polysaccharides tend to create helical structures whereas structural polysaccharides are automatically formed into extended formations with steric restrictions on the rotation of monomers. The composition of cellulose was established in 1838 by French chemist Anselme Payen who estimated that it consists of 44-45% carbon, 6-6.5% hydrogen and oxygen giving it the formula $C_6H_{10}O_6$ (Granström, 2009). The macromolecular chain structure was proposed by Haworth in the late 1920s and later proven by Staudinger. The D-glucopyranose units consist of six-membered rings with five carbons and one oxygen atom in the lowest energy state $^4C_1$-chair configuration linked together by β-1,4-glycosidic bonds (Rojas, 2016). A ring containing three reactive hydroxyl groups in position C6, C2 and C3 can be defined as an anhydroglucose unit (AGU). Bonding causes every other ring to rotate 180° around the chain axis forming a linear syndiotactic homopolymer as illustrated in Figure 2. High crystallinity, limited solubility and high reactivity of the hydroxyl groups are the results of strong hydrogen bonds.

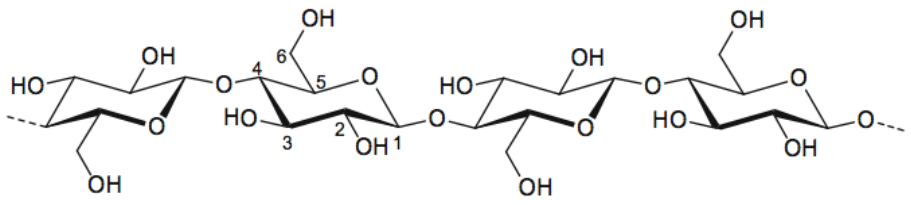


Figure 2: Cellulose polymer chain configuration. A primary reactive hydroxyl group is located at C6 and two secondary groups at C2 and C3 (Rojas, 2016).

There are four existing polymorphs of cellulose of which crystalline native cellulose, cellulose I is most common and appears in lignocellulosic plants (Klemm et al. 2015, Dufresne, 2018). The cellulose macromolecules are not evenly distributed throughout the fibril structure and can be divided into regions of so-called amorphous parts with low crystallinity and high order crystalline regions (Klemm et al. 2015).

2.1.1 Nanocellulose

According to a definition set by the European Commission in October 2011, materials in which 50% or more of the particles have at least one outer dimension smaller than 100nm are classed as nanomaterials (European commission, 2017, Kangas, 2013). The definition for nanocelluloses has been broad since the production was introduced in the late 1970s, but standards have since then been proposed and some abbreviations made more popular (Kangas, 2013, Rissanen, 2017). Nanocellulose from vegetal sources can be categorized based on manufacturing processes and dimensions and some of the existing abbreviations and synonyms are shown in Figure 3 (Zimmermann et al. 2016). In this work cellulose nanofibrils are denoted as CNF and cellulose nanocrystals as CNC.

Table 1:Nanocellulose abbreviations (Zimmermann et al. 2016)

Processes	Abbreviations	Synonyms
Mechanic	**CNF**, MFC, NFC	**Cellulose nanofibrils**, nanofibrillated cellulose, cellulose microfibers, fibrillated cellulose.
Chemical	**CNC**, CNW, NCC	**Cellulose nanocrystals**, whiskers, crystal of cellulose, crystalline nanocellulose.

2.1.2 Cellulose nanofibrils (CNF)

In 1983 it was discovered that the viscosity of softwood pulp increased as it was passed through a homogenizer multiple times (Tardy et al. 2017). CNFs generally isolated using top down methods without any enzymatic or chemical treatments have large property variations in width and length depending on isolation methods, processing intensity and

source. This makes characterization difficult (Rissanen, 2017). The CNFs consist of bundled elementary fibrils with widths typically between 20-50 nm and lengths of 500-2000 nm (Kargarzadeh et al. 2017). The crystallinity ranges from 60 to 70% and they have a specific surface area of 100-200 m^2g^{-1} (Rissanen, 2017). The advantage of CNF compared to CNC and bacterial cellulose (BC) is that it is relatively easily produced in industrial quantities and can be utilized in a wider range of applications (Naderi, 2017). The nature of CNF allows it to be used as a reinforcement in composites, paper and board, rheology modifier for example in cosmetics, as an additive in foodstuffs or to improve barrier properties in packaging (Kangas, 2013). Fully green packaging materials is a promising application for CNF as it can self-assemble to forms dense films with good barrier properties (Österberg et al. 2013).

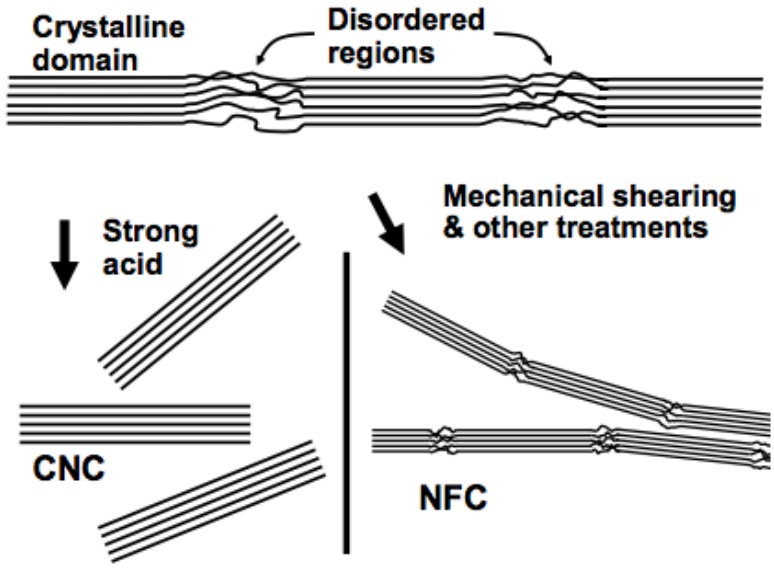


Figure 3: Visual representation of CNC and CNF (Hubbe et al. 2017)

2.1.3 Cellulose Nanocrystals (CNC)

It was not until acid hydrolysis was introduced that crystalline regions could be isolated from fibers (Koon-Yang, 2018). By selective degradation the amorphous regions are removed using strong acids leaving the highly ordered sections intact. Cellulose derived from biomass are typically 25 to 75% crystalline and the crystallinity of isolated CNCs is below 80% (Tardy et al. 2017). These highly crystalline regions possess incredible strength and rigidity up to the theoretical modulus of a perfect crystal (138-150 GPa) and

transferring these to a composite would yield immense load bearing mechanical properties (Ng et al. 2015). The proposed surface area of CNCs is 150-250 m^2g^{-1} (Kangas, 2013). According to Koon-Yang (2018) most of the research community agrees on a misconception about the nature and ratio of crystalline and amorphous regions in cellulose nanofibrils. This collective argues that due to their widely different properties and frequency of the unordered regions, the amorphous parts could be seen as defects in the crystallites. Koon-Yang (2017) argues that amorphous regions in ramie fibers which only span about four to five AGU (1.5% of the total mass) are too small to be compared with bulky amorphous regions in synthetic semi-crystalline polymers.

2.1.4 Bacterial nanocellulose (BC)

A bottom-up nanocellulose production method utilizes bacteria that synthesize chemically pure cellulose, and it is this purity that makes BC appealing compared to plant cellulose. For commercial applications only the *Acetobacter* species has been observed to produce large enough quantities of cellulose. This impressive non-photosynthetic organism that appears in the fermentation process of sugars and plants can convert ethanol to acetic acid and convert sugars into cellulose (Dufresne, 2018). The cellulose synthesis is believed to offer protection from external threats and work as a flotation device ensuring the availability of oxygen which is vital for the organism. Other speculative functions include protection from UV-light and acting as moisture absorbent. The most studied Acetobacter species *Acetobacter xylinum* which procures sugars also goes by the name of *Gluconacetobacter*. It can be bought from international collections of micro-organisms (Dufresne, 2018).

Table 2: Cellulose producing species of bacteria (Dufresne, 2018).

Genus	Cellulose structure
Acetobacter	Extracellular pellicle composed of ribbons
Achromobacter	Fibrils
Aerobacter	Fibrils
Agrobacterium	Short fibrils
Alcaligenes	Fibrils
Pseudomonas	No distinct fibrils
Rhibozium	Short fibrils
Sarcina	Amorphous cellulose
Zoogloea	Not well defined

2.2 Production of cellulose nanofibrils

2.2.1 Purification

The starting point in the production process depends on the raw material that is to be used. Often bleached pulps are chosen to eliminate the step of removing unwanted non-cellulosic impurities (Dufresne, 2018). If raw fibers are chosen as starting material, a source dependent chemical treatment is required. In a general approach described by Dufresne (2018) the raw biomass is ground to increase surface area and maximize accessibility and waxes are occasionally removed using a soxhlet apparatus with a solvent mixture. Following the wax removal step, an alkali treatment using 2% NaOH solution at 80 °C is used to remove water-soluble polysaccharides. After washing and filtering out the by-products the bleaching step is done using Sodium chlorite ($NaClO_2$) to remove the last polysaccharides e.g. lignin, proteins and polyphenols. This procedure should yield a material simply consisting of the cellular structure (Dufresne, 2018).

2.2.2 Chemical and enzymatic treatments

Nature inspired enzymatic and chemical pretreatments can be used to reduce the bonding energy before attempting to mechanically isolate the elementary fibers (Missoim et al. 2013). In most chemistry assisted processes, the fibrillation of wood, cotton, bacterial and tunicate celluloses is assisted by introducing anionically charged functional groups to form repulsion between the water and fibrils. An efficient treatment known as tempo-

mediated oxidation and TEMPO is an abbreviation for the use of 2,2,6,6-tetramethylpiperidine-1-oxyl radicals. This process isolates elementary nanofibrils from native wood fibers using nitroxyl radicals simultaneously changing the surface chemistry by selective conversion of C6 primary hydroxyls to carboxylate groups (Isogai et al, 2010). These commercially available nitroxyl radicals mostly have a negative result on the Ames test, which shows the mutagenic activity. This is important as it can be used to safely manufacture a wide range of consumer products. Enzymes effectively degrade cellulose in nature and fibers with well-preserved molecular weight and length have been obtained using controlled disintegration at very low enzyme concentrations (Missoim et al. 2013). Acid hydrolysis treatments significantly reduce the length of fibers by dissolving amorphous regions and excessive hydrolysis results in short, low molecular weight CNCs (Henriksson, 2007).

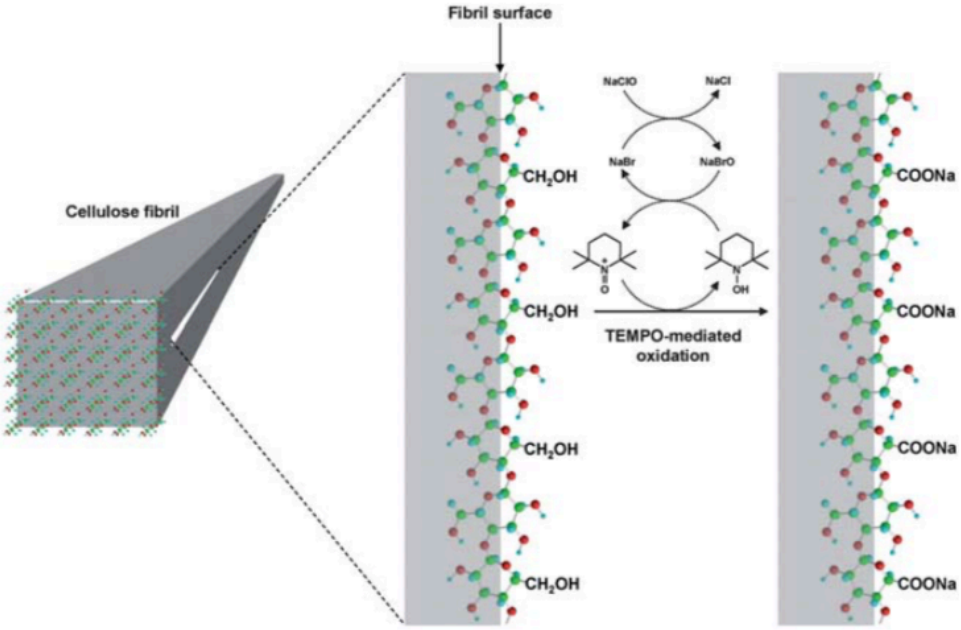


Figure 4: Oxidation of cellulose primary C6 hydroxyls to C6 carboxylate groups using TEMPO/NaBr/$NaClO_2$ (Isogai et al 2010).

2.2.3 Mechanical treatment

Top-down methods work by delamination of cellulose microfibers to obtain CNF (Ding et al. 2018). The energy demanding mechanical treatments apply shear forces of large magnitude to break intermolecular hydrogen bonds and split the microfibrils into substructures seen in Figure 1 (Dufresne, 2018). Isolation of elementary fibrils is strenuous

and not conceivable without substantial mass losses (Isogai et al. 2010). The treatment may damage the structure, reduce the crystallinity or completely fail to disintegrate the fibers (Henriksson et al. 2007). The degree of polymerization (DP) of top-down isolated cellulose is between 800 and 3000 (Rojas, 2016). Mechanical fibrillation is most commonly done by one, or in some combination of the three following methods: homogenization, grinding or microfluidization, but it can also be done purely by chemical treatment, high-intensity ultrasonification processes or chemical/enzymatic treatments followed by mechanical fibrillation (Ding, 2018, Khalil et al. 2012). Chemically assisted mechanical fibrillation has a significantly lower energy consumption than pure mechanical treatments (Rissanen, 2017). Traditional high-pressure homogenization processes developed in the 1980s work by forcing an aqueous suspension through a slit subjecting the cellulose fibers to shear forces, pressure differences, cavitations and shockwaves illustrated in Figure 5 (Rissanen, 2016, Kargarzadeh et al. 2017). As the suspension is forced through the slit, the velocity and therefore dynamic pressure increases; but static pressure drops. This drop below the vapor phase of water causes gas bubbles to form which then collapse when reaching normal air pressure. A viscosity dependent gap width of 5 to 20 µm is used and the pressure reaches 50-2000 MPa (Kargarzadeh et al. 2017). For the suspension to reach adequate fibrillation several passes through the homogenizer are required, this also increases the energy consumption (Henriksson, 2007). The relative complex machinery can cause clogging adding disadvantages to the already energy and time-consuming process (Kangas, 2013). Kargarzadeh et al. (2017) point out that excessive processing intensity causes degradation of the crystalline structure.

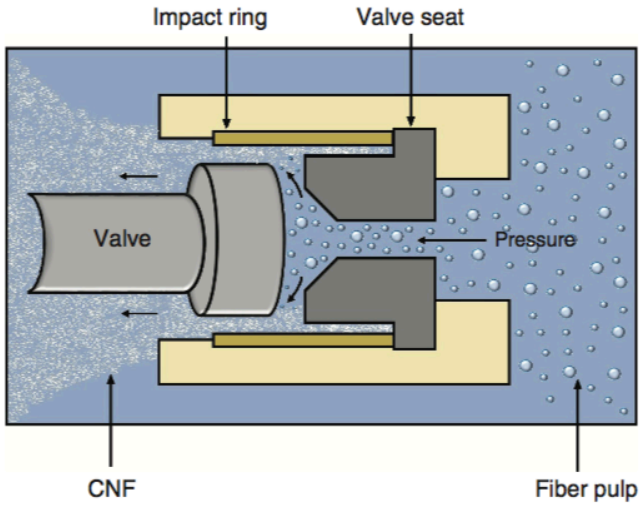


Figure 5: Homogenizing process of fiber pulp to reduce particle size. The large particle solution is pumped from the right through the valve where the particle size is reduced (Kargarzadeh et al. 2017).

Microfluidizers operate at a constant shear rate by injecting the suspension into a narrow channel with sharp turns (often z-shaped) breaking down the fibers on impact with the channel walls (Figure 6). The machine architecture allows circulation of the suspension to maximize efficiency (Kargarzadeh et al. 2017). Rissanen (2018) points out that this is more efficient and requires a smaller amount of cycles (5-20 cycles) than the conventional homogenization process and that the absence of moving parts also lowers the probability of clogging.

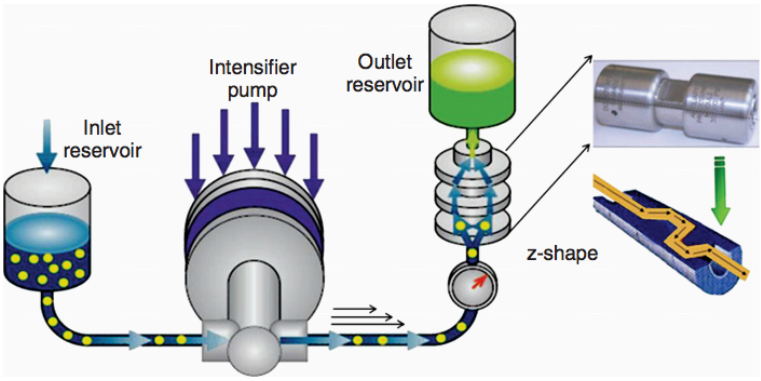


Figure 6: Microfluidics microfluidizer process (Missoum et al. 2013)

Grinders utilize grindstones shown in Figure 7 to mechanically fibrillate cellulose (Missoim et al. 2013). The two grindstones rotate at a speed around 1500 rpm relatively to each other and the resulting particle size is dependent on the adjustable distance between stones (Kargarzadeh et al. 2017). Water evaportation caused by friction makes keeping track of pulp consistency difficult as the process needs to be repeated many times.

Figure 7: Masuko high-intensity grinder. Machine is fed from the top. Rough grindstone shown in bottom right corner. (Missoum et al. 2013)

2.2.4 Drying techniques

Removing liquids is necessary for preparing a majority of CNF composites, this raises a problem as most cellulose processing is done in aqueous suspensions (Zimmermann et al. 2016). Two common drying methods are evaporation and lyophilization. The evaporation method can be divided into three steps according to Zimmermann et al. (2016): A constant evaporation stage after which there is a decline in drying rate. The second stage as the fibers start to have limited mobility and vapor starts to diffuse inside the suspension. The final step is when the distance between particles becomes small and submolecular interactions occur. As the particles are brought together, irreversible and partly reversible intermolecular hydrogen bonds form due to reactive hydroxyl groups. This process is called hornification and has a long history in the paper and pulping industry (Diniz et al. 2004). These agglomerations of fibers are difficult to separate, and the suspension will not retain the initial swollen state once it has been dried. The second and simplest ways of removing water without impairing the structure and volume is lyophilization, which also is called freeze-drying (Zimmermann et al. 2016). This works by freezing the aqueous suspension and then removing the water by directly converting it from its frozen state to water vapor at pressures below the triple point of water and temperatures between -20 °C and -50 °C.

2.3 Modification of nanofibrillated cellulose

The hydrophilic nature of cellulose caused by hydroxyl groups is problematic in many cases. Surface modifications are appealing because of a number of reasons; e.g. Drying without forming aggregations and improving compatibility with a wider array of matrix materials. Chemical modification of the hydrophilic hydroxyl groups remains a prominent strategy to minimize surface polarity to allow uniform dispersion in nonpolar mediums (Abdulkhani et al. 2014). However, a too large degree of substitution can negate the desired percolation effects “insulating” the components from each other (Capadona et al. 2007). Some common modification techniques used to increase the hydrophobicity of cellulose are discussed in this section.

2.3.1 Chemical grafting

Esterification processes using acid anhydrides, carboxylic acid or acid chlorides as reacting agents increases the hydrophobicity of cellulose and has been proven successful in improving the dispersion of CNF and CNC in nonpolar mediums, significantly lowering the amount of aggregates (Bulota et al. 2012, Missoim et al. 2013). Acetylation can be done before or after mechanical fibril isolation and pre-isolation treatment shows no significant negative impact on the degree of substitution (DS) (Jonoobi et al. 2010). Knowing this, performing the modification on wood pulp before fibrillation is more efficient from a production point of view (Oksman et al. 2016). The acetylation reaction using acetic anhydride results in esterification of the primary hydroxyl groups which hinders intermolecular hydrogen bonding (Figure 8). Due to strong hydrogen bonds between hydroxyl groups the reactivity is expected to be low (Bulota et al. 2012, Jonoobi et al. 2010). This reaction is also used when preparing ester derivatives such as cellulose acetate (Missoim et al. 2013). Acetyl reagents tend to induce swelling of the cellulose fibers exposing a larger surface area and accelerating the process. Some authors attempt to "activate" the surface before commencing (Abdulkhani et al, 2014).

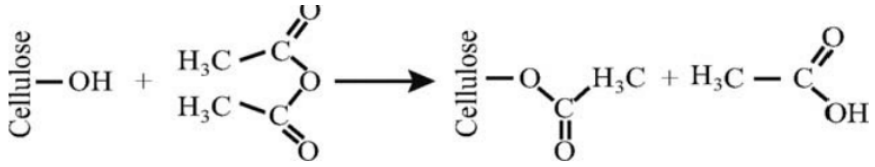


Figure 8: Acetic anhydride reaction with a primary hydroxyl group (Bulota et al. 2012)

2.3.2 Surfactants

The surface chemistry of CNC and CNF depends heavily on what isolation methods are used (Tardy et al. 2017). For example, a negative surface charge is imparted by TEMPO meditated oxidation as well as sulfuric acid hydrolysis, additionally the cellulose may contain residual anionic groups from the cell wall. The affinity between cellulose and surfactant is dependent on electric charges, hydration, solvency and surfactants used. The negative zeta-potentials on both counterparts should make the use of anionic surfactants such as alkyl sulfates and carboxylates ineffective but still some anionic surfactants tend to adsorb onto negative bearing cellulose groups (Tardy et al. 2017). It has been reported

that anionic surfactant adsorption reaches half saturation in 10 minutes onto cotton and increases with temperature. However, it is not sufficient to compete with that of cationic surfactants at cellulose/water interfaces. Tardy et al. (2017) points out that this area of complex interactions has endless possibilities that remain to be explored.

2.4 Properties of Nanocellulose fibrils and Polylactic Acid (PLA)

Some important properties och CNF and PLA are discussed in this section.

2.4.1 Thermal stability

Nanocellulose can be used to alternate the thermal characteristics of polymers. Thermal degradation of cellulose cleaves the glycosidic bonds producing glucose and releasing free radicals. Cellulose is mainly responsible for producing volatile products when burning wood (Dufresne, 2018). Above 200 °C the DP is continuously reduced forming carbonyl, carboxyl and hydroperoxide groups (Dufresne, 2018). Degradation also takes place by decarboxylation, transglycosylation, oxidation reactions and is accelerated by water, acids and oxygen (Dufresne, 2018). The physical form and surface chemistry influences the temperature and rate of thermal degradation. In an attempt to compare thermal characteristics degradation of TEMPO-oxidized CNF started at 300 °C in a nitrogen atmosphere while degradation of untreated CNF started at 200 °C (Dufresne, 2018).

2.4.2 Characterization

To determine the characteristics of nanocelluloses several methods can be utilized. According to Kangas et al. (2013) the best methods for determining its physical properties are microscopy, transmittance analysis and rheology measurement (Rissanen, 2017). Rheology measurement is a common approach as CNF is most commonly stored in liquid suspensions, typically water. To prevent clogging of machinery and facilitate handling the suspensions are kept below 2 wt% cellulose (Dufresne, 2018). Due to the large surface area, amount of reactive hydroxyl groups and increased Einstein coefficient (Increasing length to diameter ratio) CNF suspensions become gel-like in an aqueous phase already at 0.125% and the viscosity increases exponentially with increasing CNF percentage (Dufresne, 2018, Kangas, 2013). This gelling behavior is demonstrated in Figure 9.

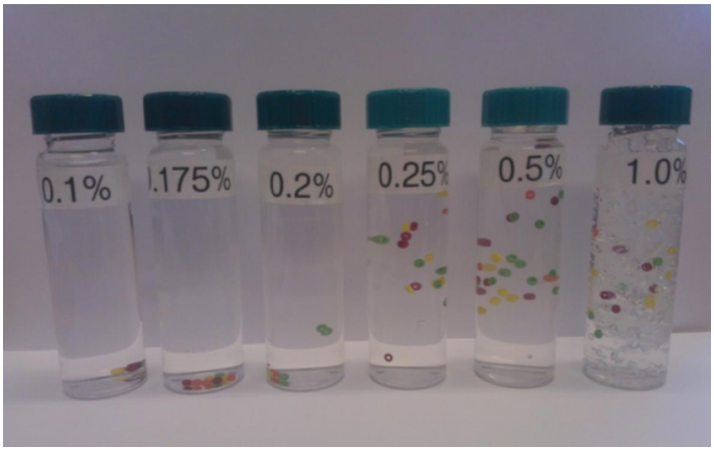


Figure 9: Visual of CNF ability to form gel-like suspensions in water at low concentrations (Kangas, 2013)

2.4.3 Shelf life

Cellulose should be treated as any other biomass and the shelf life can be extended when handled correctly. For aqueous solutions, preservability is mainly a function of temperature meaning it can be stored longer in the refrigerator. For even longer storage times freezing directly after production is recommended. Touching and contamination should be avoided as degradation and microbial growth affects viscosity, strength, reactivity and other CNF properties. For demanding applications, it is recommended to use the material within three months of treatment (Kangas 2013).

2.4.4 Polylactic acid (PLA)

Polymers originating from a biological source also referred to as biodegradable polymers are a promising replacement for many petrochemical polymers. They are challenging to work with as there always is a source dependent variance in properties (Vijay et al, 2018). Most biopolymers degrade into gases (CO_2, N_2), biomass, water and inorganic and organic salts by biological processes (Vijay et al. 2018**)**. It is worth noting that some petrochemical polymers will biodegrade and that not all bioderived polymers will (Zhu et al. 2016). Polylactic acid (PLA) is considered to be a promising candidate for replacing petrol-based polymers as it has comparable characteristics and the production has been developed significantly making it commercially available in large volumes at competitive prices (Ding et al. 2015). Derived from renewable sources such as corn, sugar beet and

cassava. For this work, PLA derived from starch was used. In manufacturing it is by hydrolysis converted into dextrose, a form of glucose, which is then fermented to lactic acid which after esterification creates the final product shown in figure 10 (NatureWorks, 2012). PLA is compostable and hydrolyzes into acid and alcohol. This process is accelerated by temperature, moisture and present acids and bases (Fukushima et al. 2009). The thermoplastic aliphatic polyester is nonpolar. Due to a slow crystallization rate and low service temperature organic and inorganic fillers, nucleating agents and other strategies to improve the crystallization kinetics of biodegradable polymers are often used (Ding et al. 2015, Ding et al. 2018).

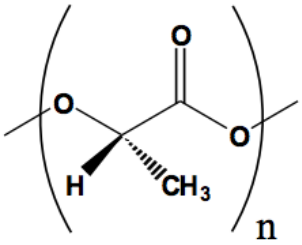


Figure 10: PLA momoner unit (NatureWorks, 2012)

2.5 Applications for nanofibrillated cellulose

Due to the relatively efficient processing methods made available allowing production of large volumes of CNF, there is a possibility for using it in a wide variety of applications (Rissanen, 2017). Laboratory scale and commercial projects are constantly developed for potential markets with high expectations. These are a wide range of both large-scale commodity, low volume and novel applications some of which are listed in table 3 (RISE, 2015). In the recent years there has been a remarkable increase in the use of CNF in packaging because of its ability to form dense networks with good barrier properties, especially high oxygen permeation resistance when compared to polymeric materials (Hubbe et al. 2017). This can be effectively applied in thin film applications, as a coating or intermediate layer. All nanocellulose applications have obvious challenges and for some areas there is great motivation and proposed methods for overcoming them. Considering the ability of cellulose to self-assemble it can be used independently in a wide variety of applications. In this work the application potential of CNFs as a filler in polylactic acid is explored. Green nanofillers are attractive the sake of biodegradability, but

also because of their low-cost and high specific mechanical properties (Jonoobi et al. 2012).

Table 3: CNF application potential (Rissanen, 2017)

High volume	**Low volume**	**Novel and emerging**
Automotive	Wallboard facing	Reinforcement fibre
Cement	Insulation	Filtration and purification
Paper coatings	Aerospace	Rheology modifiers
Paper filler	Aerogels	Cosmetics
Plastic replacement	Paints	Medical applications
Hygiene products		Flexible electronics
Textiles for clothing		3D printing

2.5.1 Composites

Composites are combinations of two or more materials with different phases and constituents (Vijay, 2018). Conventionally a lower modulus, continuous phase (matrix) is used in combination with a dispersed reinforcing phase (filler) to obtain a combination with overall increased performance (Vijay, 2018). Composites can be divided into three groups (Swain et al. 2018). The first type utilizes a particle reinforcement suspended in a matrix. The second is commonly known as structural composites or sandwich composites which utilize different phases bonded to each other in layers. Lastly, the third is fiber reinforced composites that utilize long fibers embedded into a matrix. A filler material is intentionally added to alter the matrix properties, for example weight, electrical conductivity, strength and even price. It is important to note that all fillers have an impact on the mechanical properties of polymeric materials (Osswald, Menges, 2003). The main variance in composite properties is a result of processing methods as dispersion, distribution and alignment of particles depict its final characteristics (Oksman et al. 2016). In nanocomposites, nanoparticles are ideally distributed evenly throughout the composite, but lack of methods for controlling this behaviour is of concern (Capadona et al. 2007).

2.5.2 Nanofibrillated cellulose as reinforcement

The natural structural polymer with a large surface area and high strength is often viewed as the next generation reinforcement for high performance green composites (Lee et al. 2014). The abundance of methods for producing CNF and CNC on the laboratory scale has led to a large amount of publications on composites (Lee et al. 2014). According to Dufresne (2018) combining conventional high modulus reinforcements with a polymer matrix results in an improved modulus and strength of the composite but lowers the overall impact strength and ductility. Compared to the conventional reinforcements using homogenously dispersed nanoparticles connected in a three-dimensional network reduces stress concentrations and can improve strength without suffering ductility and impact strength losses. The greatest reinforcement effects on thermoplastics have been observed at small amounts of CNF when the CNF is able to form a percolated network. The threshold for the desired percolation effect is estimated to be 1-6 vol% (Lee et al. 2014). It appears that at high amounts, bonding between elementary fibrils tend to prevent the impregnation of polymers into the dense cellulose networks (Lee et al. 2014). Another implication for composites produced by solvent casting unmodified CNF with homogenous dispersion is a wider glass transition range and higher strength above the glass transition temperature (Kargarzadeh et al. 2017, Murphy & Collins, 2016). The CNF surface area of 100–200 m^2g^{-1} is immense when compared to glass and natural fibers with surface areas below 10 m^2g^{-1}. Utilizing the contact area is key for transferring the properties from reinforcement to matrix (Rissanen, 2017, Sabu et al. 2013). The prediction and modelling of nanocomposite mechanical properties materials is difficult for fibrillated nanocellulose due to its inconsistent morphology, tangling and flexible nature. Especially when compared to the simplicity of the CNC rod-shaped particles (Dufresne, 2018).

2.5.3 Types of nanocellulose reinforced composites

Nanocellulose was used as a filler in thermoplastic polymer matrices for the first time in 1987 by Boldizar et al. but the full reinforcing effect of small amounts was not apparent until 1995 when Favier et al. used cellulose microfibrils in polymer matrices (Lee et al. 2014). Following this, cellulose was used in hydrophilic elastomers and water-soluble polymers such as potato starch because of easy dispersion in aqueous suspensions and

large reinforcing potential (Lee et al. 2014). For these applications polymers with functional groups allowing water solubility can be dissolved and mixed directly with an aqueous cellulose suspension eliminating the need for complex compounding processes, solvent exchange procedures and drying steps (Dufresne, 2018). After mixing the two components, water can be evaporated, filtered or the whole mixture freeze dried and hot pressed. The polar nature of both constituents permits a strong interaction between the reinforcement and matrix (Oksman et al. 2016). In 2005 another approach to avoid the dispersion problem and significantly improve mechanical properties was developed. This was done by producing nanopapers which were impregnated with thermosetting polymers (Oksman et al. 2016). Nanopapers can be used as 2D reinforcements with predictable properties in laminated composites (Mautner et al. 2017).

2.5.4 Polylactic acid nanofibrillated cellulose composites

One major obstacle to creating PLA/CNF composites is avoiding flocculation as the hydrophilic cellulose does not pair well with the hydrophobic matrix material (Murphy & Collins, 2016). The polar cellulose fibrils can cause serious dispersion- and compatibility problems in non-polar mediums and require some modification to increase hydrophobicity and improve interfacial adhesion to the matrix (Khalil et al., 2014). Contradictive material properties which prevent adequate dispersion also cause the long fibrils to agglomerate reducing surface area and lowering mechanical performance. Lignocellulosic fibers usually improve the tensile properties of PLA, compromising impact strength and making it more brittle. This can be explained by the lacking interaction between fiber and matrix (Immonen, et al. 2017). Acetylation is seen as being a promising alternative reducing the CNFs affinity to water and improving its compatibility with nonpolar solvents, matrices (Tingaut et al. 2010). Often the acetylation is done by subjecting the CNF to a large excess of acetic anhydride to maximize the DS, after it is washed and solvent exchanged with desired solvents. Tingaut et al. (2010) showed that the amounts of aggregates decreased, and the films became increasingly transparent as the DS increased (Figure 11). The mechanical strength however was significantly greater for that of unmodified fibers than acetylated fibers. The DS can be measured using FTIR spectroscophy (Tingaout et al. 2010). Contradicting results have been reported for the use of nanocelluloses as nucleating agents. Ding et al. (2015) showed a significantly higher crystallization

rate in PLA/CNF composites compared to neat PLA. In that study the nucleation power also increased as a function of CNF content.

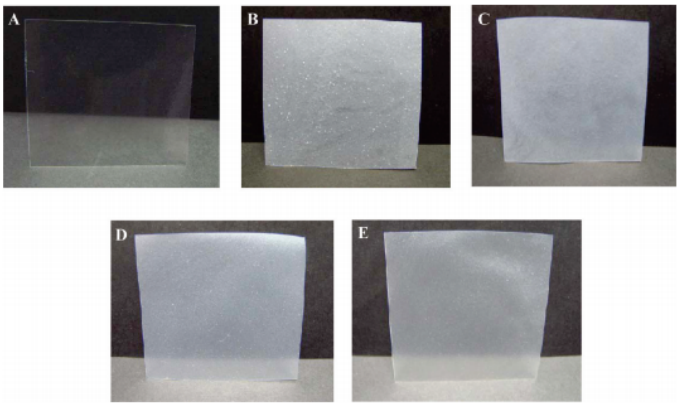


Figure 11: PLA and PLA/acetylized films produced by Tinagut et al. (2010). (A) Neat PLA (B) 10 wt% unmodified CNF (C) 10 wt% acetylated CNF with DS 3,5% (D) 10 wt% acetylated CNF with DS 8,5% (E) 10 wt% acetylated CNF with DS 17%

2.6 Methods for producing cellulose nanocomposites

In the process of mixing CNF into a matrix reaching adequate dispersion is key to forming a continuous nanoparticle network of single fibrils (Dufresne, 2018, Capadona et al. 2007). Most solvent casting methods allow preservation of the dispersion state and based on quantitative analysis solution casted samples have the highest mechanical properties that can be reached for a given polymeric system (Dufresne, 2018). Due to the relatively slow evaporation rate and Brownian motion particles have time to rearrange and form a network. Good dispersion has been achieved using melt compounding by using a dual screw extruder rotating in opposites directions (Oksman et al. 2016). Two common composite production methods are reviewed in this section.

2.6.1 Solvent casting

A common approach to dispersing particles in nanocomposites is using solvents. By either doing the process as a solvent mixture or solvent transfer. Solvent mixtures require a polymer solution with a water miscible solvent for suspending the particles. Processing can, for example, be done using compression molding and then evaporating the solvent mixture.

In a solvent exchange process the CNF is transferred to and suspended in a solvent. This process also allows the liquid suspension to be used as a medium for further surface modification of the nanoparticles (Dufresne, 2018). By dissolving the polymer matrix and directly mixing the particles suspended in solvent into the polymer solution yields a suspension with particle dispersion greater than that of mixing particles into a polymer melt (Scruben et al. 2000). For casting, a smooth surface such as a glass plate or PTFE mold cleaned with acetone should be used. Sceirs (2000) advised not to use release coatings as it may lead to artifacts in the analysis stage. After pouring the polymer solution into the mold, the solvent is evaporated. The appearance and texture of composites produced by solvent casting can be different depending on the evaporation rate (Sceirs, 2000).

In laboratory scale production this method has been used instead of a dry compounding stage for reaching adequate dispersion. Murphy and Collins (2016) produced PLA/microcrystalline cellulose filament by solvent casting films which were then ground and extruded using a twin-screw extruder. In this case processing temperatures of 165–190 °C were used relying on that the cellulose will not undergo thermal degradation. The 3 and 5 wt% CNF/PLA films with both modified and natural fibers experienced a wider glass transition range and higher strength.

2.6.2 Melt compounding

Melt processing is important to the industry because it is simple, cheap and scalable. Depending on production scale, the melts can be processed either in batches or extruded continuously (Ding et al. 2018). For laboratory scale work and pilot projects microextruders are preferred (Oksman et al. 2016). It is worth noting that microextruders have long processing times which often lead to degradation of either cellulose or the matrix polymer. The nanocellulose can be introduced to the polymer melt in the extrusion process either in dried form or in a liquid suspension each of which have conceptual drawbacks and advantages. Extruders commonly operate as closed systems resulting in trapped steam and gas from the often aqueous nanocellulose suspensions. Solution feeding is more cost efficient because of the complexity of energy demanding and time consuming

drying processes. Additionally, dry fluffy fibrils obtained can be difficult to redisperse depending on modifications done to its surface chemistry (Ding et al 2018).

2.7 Characterization and testing of composites

Material testing is done to classify performance and attributes of materials. There are several methods to determine different properties of materials and the quantitative data gathered supports research and development. Some essential methods for analysis of nanocomposites are discussed in this section.

2.7.1 FTIR spectroscopy

Spectroscopy measures the interactions between radiation and materials and there are many systems available that can be used for identifying polymers and reactions. FTIR, also known as infrared spectroscopy, utilizes electromagnetic radiation in the infrared region to cause vibration and rotational changes in molecules. These distinctive vibrations help identify the chemical structure of polymers and additives (Naranjo et al. 2008).

2.7.2 Differential scanning calorimetry (DSC)

DSC allows the user to determine the thermal transition periods of polymers. This works by comparing the heat required to increase the temperature of a polymeric sample with a reference piece which does not react in the desired testing range and has accurately known transitional periods: tin and mercury are often used (Osswald, Menges, 2003). Common DSC machines operate with samples in the milligram range (<10 mg).

2.7.3 Mechanical testing

Dynamic mechanical analysis (DMA) is a suitable method for testing the mechanical behavior of materials at different temperatures (Dufresne, 2018). This allows the user to study the impact of reinforcements beyond the glass transition range of the matrix. For characterization of material tensile properties, a tensile testing machine is used (ASTM International, 2002). Results vary with sample dimensions, processing techniques, testing speed, grips used and way of measuring extension. Tensile test results provide data for

research and development as well as quality and specification purposes. A tensile testing machine has several components. Grips lined with rubber, which hold the sample, and of which one is connected to a movable member. A drive mechanism, which moves the movable member at a constant velocity set by the user. A load indicator, which shows the tensile load on the member held by the moving grips. The ultimate tensile strength can be calculated from obtained data using:

$$UTS = \frac{F}{A}$$

Where UTS is the ultimate tensile strength, F is the largest force applied and A is the nominal cross-sectional area of the specimen. Standard deviation is calculated using the formula (ASTM International, 2002):

$$s = \sqrt{\frac{(\sum X^2 - \overline{X)}}{n-1}}$$

Where s is standard deviation, X the value of one observation, n number of observations, $\bar{X}$ is the mean of the set of observations.

2.7.4 Microscopy

Optical microscopy is widely used to analyze polymers due to its simplicity and relatively low cost (Scheirs, 2000). Polarized microscopy allows the user to see defects and thoroughness of mixing; especially in fiber-reinforced semicrystalline polymers the constituents will be clearly distinguishable. In the case of CNFs the size distribution of particles should be below that of the visible range.

2.7.5 SEM

Scanning electron microscopy is a good method for analyzing the surface topography of polymers (Scheirs, 2000). Samples are mounted to a conductive substrate and grounded. Non-conductive samples must be coated to reduce charging due to accumulation of negative charges on the sample surface, this will cause bright spots to appear on the image.

3 EXPERIMENTAL WORK

3.1 Introduction

The aim of this experimental work was to produce 0.2 mm thick PLA/CNF composites with a dry CNF content of 2 and 5 wt% both using acetylated and unmodified CNF. Additionally, a neat PLA film should be made for reference. The tensile strength of neat PLA was to be compared to that of composites with acetylated and untreated fibrils. Over 17 samples were created as a part of this thesis and the process was optimized as a result of trial and error.

3.2 Materials

A 1.7 wt% aqueous suspension of native grade CNF prepared using mechanical fibrillation from bleached birch kraft pulp was obtained from VTT Technical Research Centre of Finland (Figure 12). Mechanical fibrillation was done first by grinding with a Masuko MKCA 6-2 supermasscolloider and then circulating the suspension trough a fluidizer eight times. PLA Ingeo 2003D derived from annually renewable resources from Natureworks was used as a matrix (Figure 13). PLA 2003D has a specific gravity of 1.24 and melt flow index of 6 g / 10 min (210 °C, 2.16 kg). Chloroform (Merck, Emsure and Uvasol grades), methanol (Merck, Emsure grade), glacial acetic acid (Alfa Aesar, 99%), sulfuric acid (Sigma Aldrich, 95–98%) acetic anhydride (AnalaR, 98%), ethanol (Altia, Etax A, 94%), acetone (Biltema), Chemlease 75 EZ (Chem Trend) and deionized water were used in the experiments. Petri dishes with inner dimensions of 110 mm x 19 mm and volume 180 mL were chosen to enable the production of samples with sufficient surface area and thickness as solvent evaporation methods will require the mold to accommodate large volumes of solvent to be evaporated. The experimental apparatus comprised 250 and 500 mL Schott bottles for mixing CNF/PLA/chloroform, a 10 mL Thermofisher Finnpipette with disposable tips for accurate pipetting, PTFE-coated magnetic stirrer with hot plate for mixing and heating, Finnsonic ultrasonic bath (figure 17) for homogenization, Sonics Vibra-Cell VCX 130 ultrasonic processor, VWR centrifuge (Figure 17), thermometer, laboratory stand, clamps, tubing, a 250 mL three necked bottle with plugs, reflux condenser (Figure 14) and 20 mL test tubes with plastic caps and test tube holders.

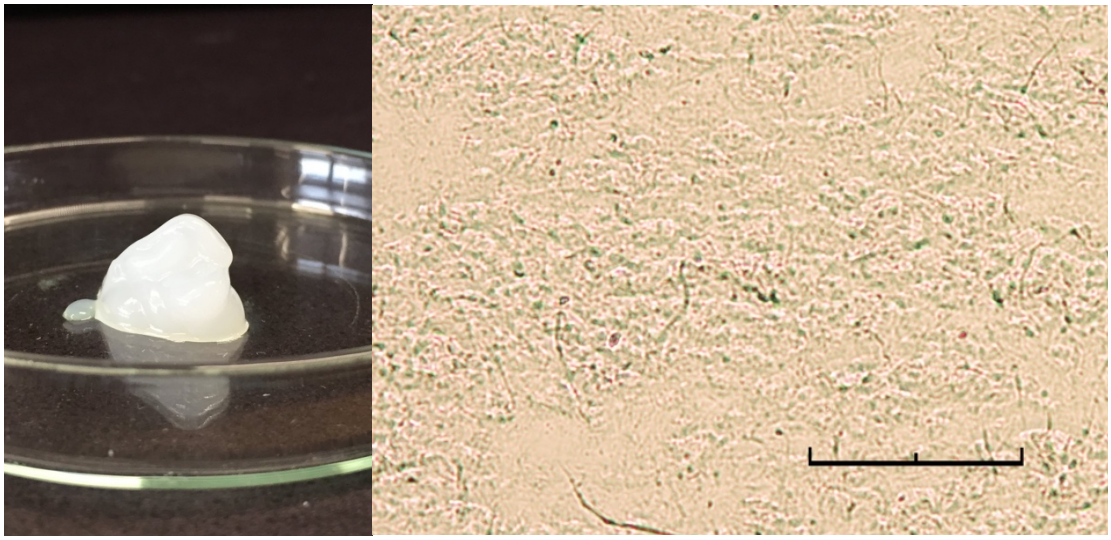


Figure 12: Left: 1,7wt% aqueous CNF suspension. Right: 20x magnification, transmitted light microscope image of CNF. Scale bar of 100μm.

3.3 Preparation of the composites

The production process included many steps and variables. In this section all of the steps for making the films are explained in chronological order.

3.3.1 Dissolution of polylactic acid

The amount of PLA needed for all samples was calculated based on film thickness restricted by the available mold volume. PLA was dried in an oven at 110 °C for 15 minutes after which the dissolution was done by measuring 500 mL (765 g) Uvasol grade chloroform into a sealable Schott-bottle (Figure 13) and adding 22.85 g of PLA. The mixture was then mixed using a magnetic stirrer for 4 h obtaining a homogenous solution. Both 2, 3 and 5 wt% PLA stock solutions were tested but the 5 wt% solution was discarded due to its high viscosity and 2 wt% due to the excessive amount of solvent needed. The use of Dichloromethane (DCM) was also explored and was proven just as effective as chloroform for dissolving PLA.

Figure 13: Left: NatureWorks Ingeo 2003D PLA granules. Right: Mixing PLA in chloroform. PLA easily sticks to the walls of the container before dissolving.

3.3.2 Chemical modification of cellulose nanofibrils

An acetylation method done by Abdulkhani et al. (2014) was used. For this, 21.6 g of the 1.7 wt% aqueous CNF suspension was used which could be used to produce four CNF/PLA films, two with 2wt% and two 5wt% dry cellulose. A reflux condenser setup with cold water flow, magnetic stirrer, hotplate and water bath seen in Figure 14 was built to prevent evaporation. A magnet was included in the water bath to prevent bumping. 25 mL acetic acid and 5 mL sulfuric acid was added to a 250 mL three necked flask containing the aqueous cellulose suspension, and the contents were heated to and kept at 70 °C for 30 minutes while stirring with a magnetic stirrer to activate the CNF surface. 50 mL of glacial acetic acid and 10 mL of acetic anhydride was added and the suspension was kept at 100 °C for 4 h. Subsequently the solution was removed from heating and left overnight. The acetylated cellulose suspension was mixed using a magnetic stirrer and weighted having a total mass of 124.95 g. 35% of this assumed homogenous suspension was measured and divided into 12 test tubes for 5wt% films and 15% to be used in 2wt% films was measured into 6 test tubes. The CNF was then isolated from the waste products using a S & C process explained in the next section.

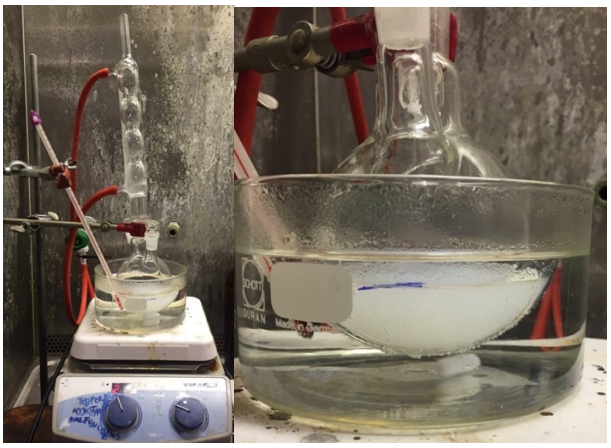


Figure 14: Reflux condenser setup

3.3.3 Solvent exchange

A solvent casting approach reported by Bulota et al. (2012) was chosen for transferring the CNF out of the aqueous phase and into chloroform. A water-miscible solvent was needed and therefore the miscibility of methanol, ethanol, acetic acid and acetone was tested by placing 0.5 g of the 1.7 wt% aqueous CNF suspension in test tubes and adding 4 g of the respective solvents. Methanol was subsequently chosen on the basis for effortlessly forming a homogenous suspension and as it has been used in earlier work in 1:2 combination with acetone (Abdulkhani et al. 2014).

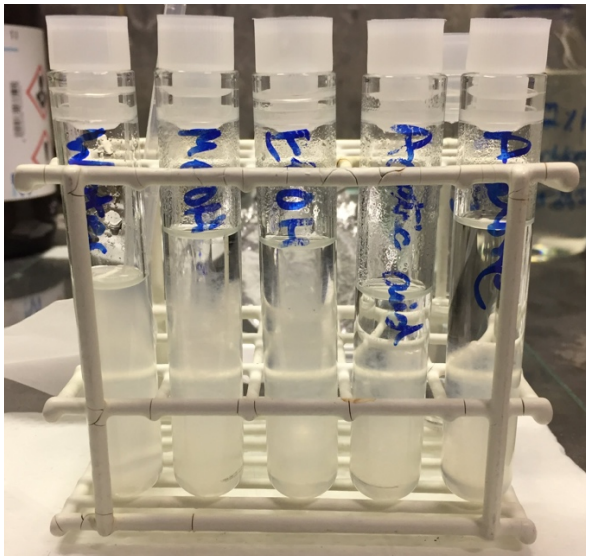


Figure 13: Aqueous CNF suspensions.

From left: Distilled water, Methanol, Ethanol, Acetic acid, Acetone.

To start the transfer process for casting test samples with 2 wt% CNF, 3.07 g of the 1.7 wt% aqueous CNF suspension was measured into a test tube and 8 g of methanol was

added. The CNF coagulated into a large lump and unsuccessful attempts to break up the agglomerations by extensive sonication both using the Sonics processor and Finnsonic ultrasonic bath showed no effect. Sonicating for extended of time periods in the Finnsonic bath also caused the water to heat up which appeared to amplify the phase separation and slow down the homogenization process. To counter this an ice pack was used to keep the water temperature around 20 °C (Figure 17). After sonicating for different periods of time for different samples it appeared that high concentration was the main issue. The amount of CNF to methanol was set below 0.2 wt% dry cellulose as the high viscosity of the gel like CNF/water/methanol suspension in combination with the limited volume of the test tube were preventing the methanol from penetrating into the aqueous CNF suspension. Dilution of the aqueous CNF suspension with distilled water was tested but the use of methanol proved just as effective and more convenient as the water would have to be removed anyway.

In the final solvent transfer process used for making the films 6 g of methanol was added to each of six test tubes containing 0.51 g of the 1.7 wt% aqueous CNF suspension. For 5 wt% CNF films 0.64 g was measured into 12 test tubes. The test tubes with acetylated cellulose in methanol prepared in section 3.3.1 were used to prepare the actylated CNF/PLA films. The samples were sealed with plastic caps, mixed by hand and then sonicated using the ultrasonic bath (Figure 17) for 10 minutes to break up agglomerates and homogenize the suspension. The Finnsonic ultrasonic bath was used as it proved to be more efficient than the Sonics ultrasonics processor and allowed many test tubes to be sonicated at a time. Following the sonication, the samples were centrifuged at 6500 rpm for 10 minutes before decanting the water and methanol phase. Subsequently 6 g of methanol was added to each test tube. This process of sonicating, centrifuging, decanting and adding methanol (S &C) was repeated 4 times as visualized in Figure 18. This process was a result of trial and error, starting out with higher CNF concentrations and shorter S & C cycles.

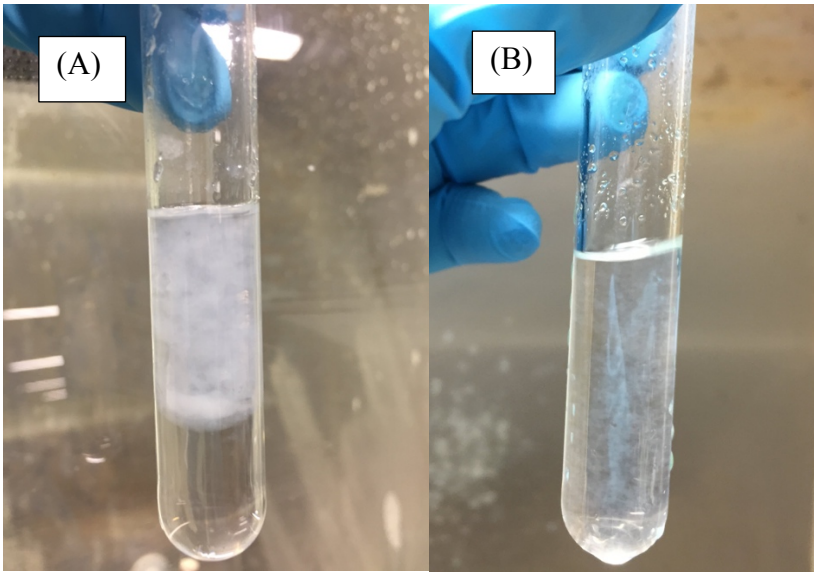


Figure 14: (A) CNF/water/methanol phase (top) and chloroform phase (bottom) separation caused by an insufficient S & C process from water to methanol. (B) CNF/water/chloroform/methanol suspension as a result of repeated sonication and centrifugation cycles.

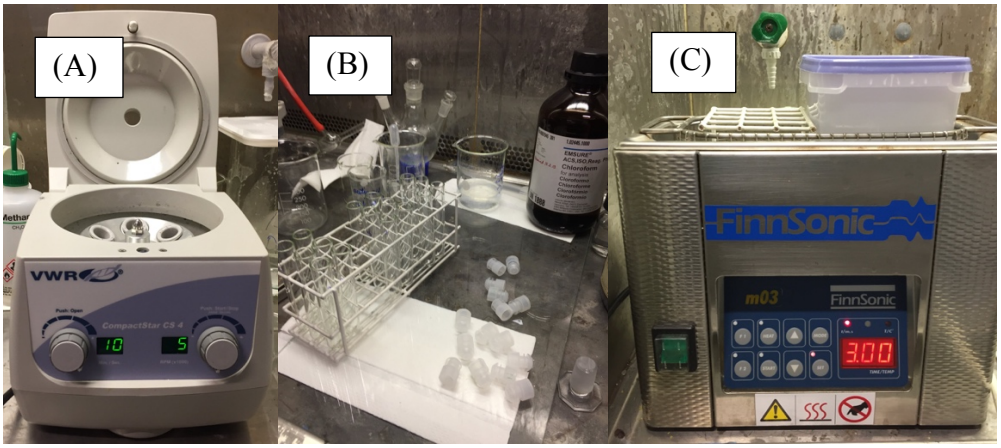


Figure 15: Equipment used in experiments. (A) VWR Centrifuge. (B) Test tubes with plastic caps. (C) Finnsonic ultrasonic bath with ice pack.

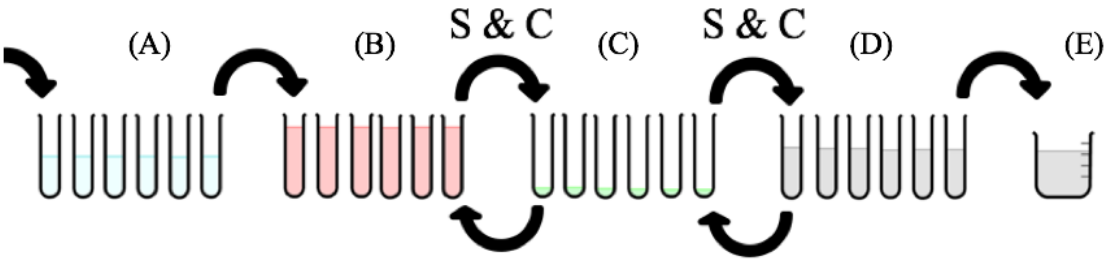


Figure 16: Solvent exchange of aqueous CNF suspension to chloroform done by a series of sonication and centrifugation (S & C) steps. (A)CNF in aqueous suspension (B) CNF/water/methanol (C) CNF (D) CNF in chloroform (E) CNF/PLA/chloroform

After the fourth S & C process 4 g of chloroform was added to each test tube. An identical sonication and centrifugation cycle was applied until the CNF did not move out of the chloroform phase in 10 minutes of centrifugation forming an even gel-like, almost transparent suspension shown in Figure 19. In the case of insufficient sonication and centrifuging into methanol, the CNF will not move into the chloroform phase as seen in Figure 16, this phenomenon is discussed in section 4.1.4. It is also worth noting that centrifuging the CNF/chloroform suspension for an excessive amount of time causes irreversible agglomerations to form on the bottom of the test tube. The samples were transferred into Schott bottles with the 3% PLA/chloroform solution (59 mL for neat PLA, 57.8 mL for 2 wt% CNF, 56 mL for 5 wt% CNF films) and the test tubes were washed with 1 mL of chloroform to minimize CNF losses. The CNF/PLA/chloroform suspensions were then mechanically mixed for 1h with a magnetic stirrer.

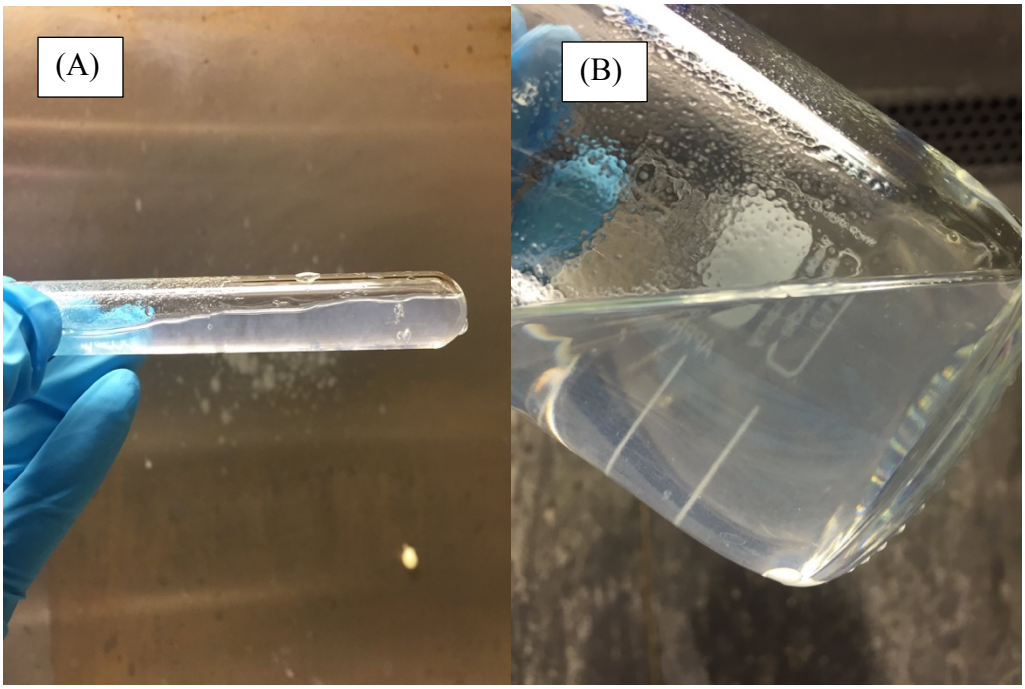


Figure 17: (A) Viscous CNF/Chloroform suspension in test tube. (B) PLA/CNF/Chloroform in Schott-bottle before casting.

3.3.4 Casting

Petri dishes were treated with Chemlease 75 release-agent four times in 15-minute intervals using lint free paper cloths. The solution was poured straight into the molds 30 minutes after the final Chemlease treatment. The molds were left in a well-ventilated fume cupboard overnight to evaporate the solvent. After evaporation the films were

peeled off from the substrates and conditioned in ambient conditions (approximately 50% relative humidity and 21 °C) for 5 days.

3.3.5 Mechanical testing

A relative measurement to evaluate the tensile strength of films was chosen and is explained in this section. The films were cut into 10 mm wide strips using a scalpel and the thickness was measured at 10 points across using a Baker K17 plunger gauge micrometer. A general tensile testing machine (Testometric M 350-5CT) with flat grips and 5 kN load cell with +/- 0,5% accuracy down to 5 N was used for testing the films (Testometric, 2018). A distance between grips of 0,041 m and speed of 20 mm/min was used and all samples were tested until break. The Wintest Testometric software allowed the student to export raw data as a text file. This data was imported into Microsoft Excel in which the ultimate tensile strengths with corresponding standard deviations were calculated and graphs were created. Youngs modulus was not calculated due to equipment load cell accuracy restrictions and weak Hookean behavior.

3.3.6 Scanning Electron Microscopy (SEM)

Representative cross-sections were obtained by fracturing the films under cryogenic condition in liquid nitrogen. Conductive double-sided carbon tape was adhered to an aluminium stub onto which was adhered the cut and fractured samples. Colloidal graphite was hand-painted on to ensure all samples were grounded in the vertical plane. The stub was then flushed with argon and sputter coated with a 5 nm thick layer of Au/Pd alloy using a Cressington sputter coater 208 HR.

4 RESULTS AND DISCUSSION

4.1.1 Results of successful and unsuccessful composite synthesis

The films produced by the developed solvent casting method are shown in Figure 20. Preliminary samples of 2wt% CNF/PLA with major defects are shown in Figure 21.

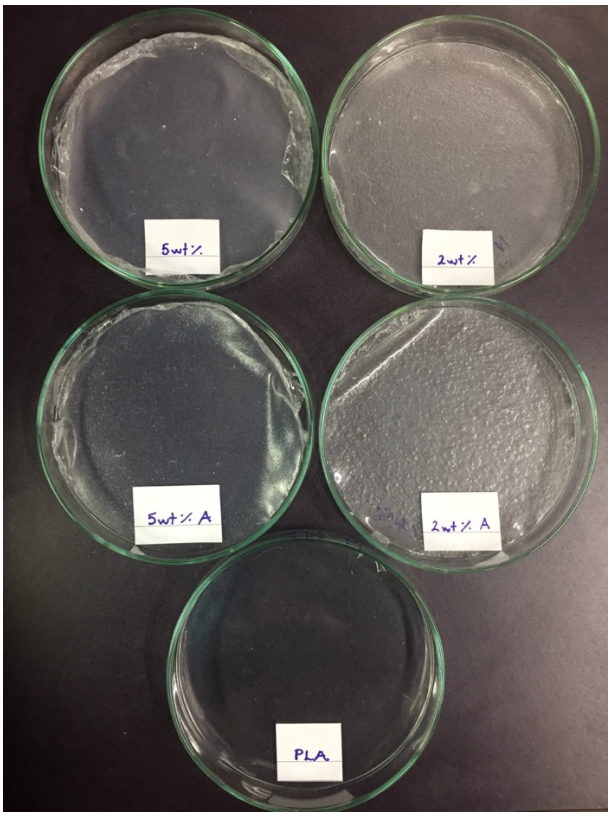


Figure 18: Produced PLA/CNF films. Top left: 5 wt% untreated CNF, top right 2 wt% untreated CNF, middle row left: 5 wt% acetylated CNF, middle row right: 2 wt% acetylated, bottom: neat PLA.

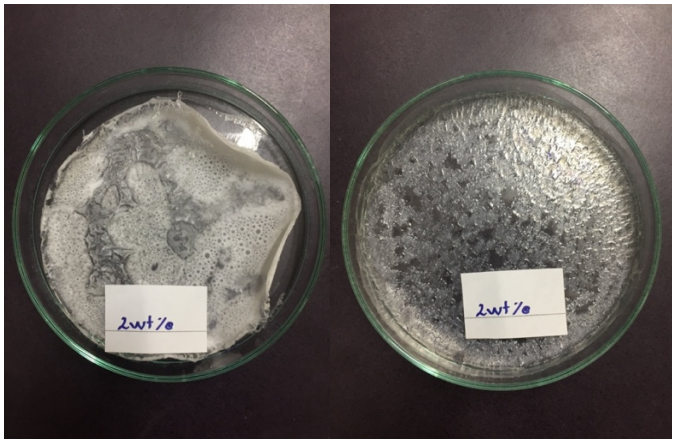


Figure 19: Early stage samples of 2wt% CNF/PLA. Left: Failure due to a high processing concentration. Right: Faults caused by not removing all water in the S & C process.

4.1.2 Results from tensile tests

The ultimate tensile strengths were calculated for all films (Figure 22). The standard deviations for each sample series (as shown by the error bars in Figure 22) are sufficiently small to conclude that the measurements were internally consistent. The data shown in Figure 23 shows the results of tensile testing with force applied on the Y-axis and elongation of the strips on the X-axis.

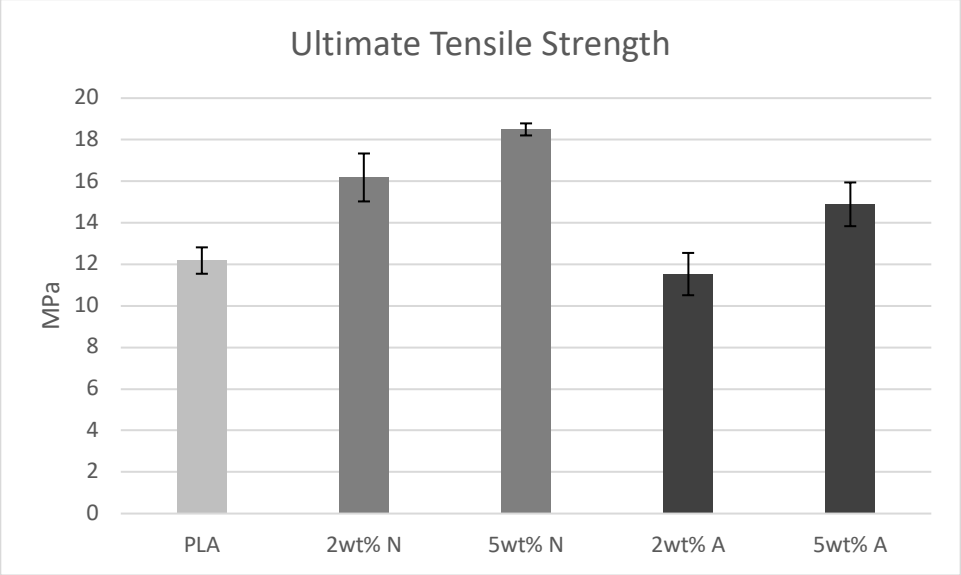


Figure 20: Ultimate tensile strength of produced films with error bar showing standard deviation. N meaning untreated CNF and A acetylated CNF.

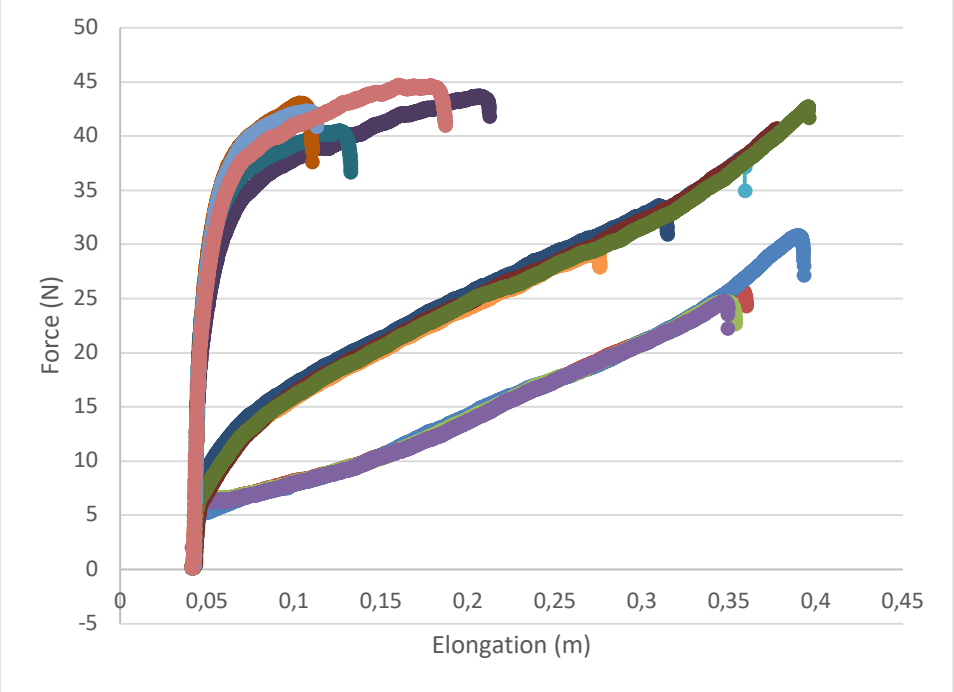


Figure 21: Tensile test results of PLA and untreated CNF/PLA

4.1.3 Results of SEM imaging

Cross sectional and top-view SEM images were taken of the samples shown in Figures 20 and 21 and analyzed. Figure 24 shows the cross section and top surface of each CNF containing film. Figure 25 shows the untreated CNF fibrils sticking out of the PLA matrix whereas Figure 26 similarly shows the cross-section of its acetylated equivalent. Figure 27 shows defects and minor irregularities on the unmodified CNF film surface. Figure 28 is a chip of the composite material found on the cross-sectional surface.

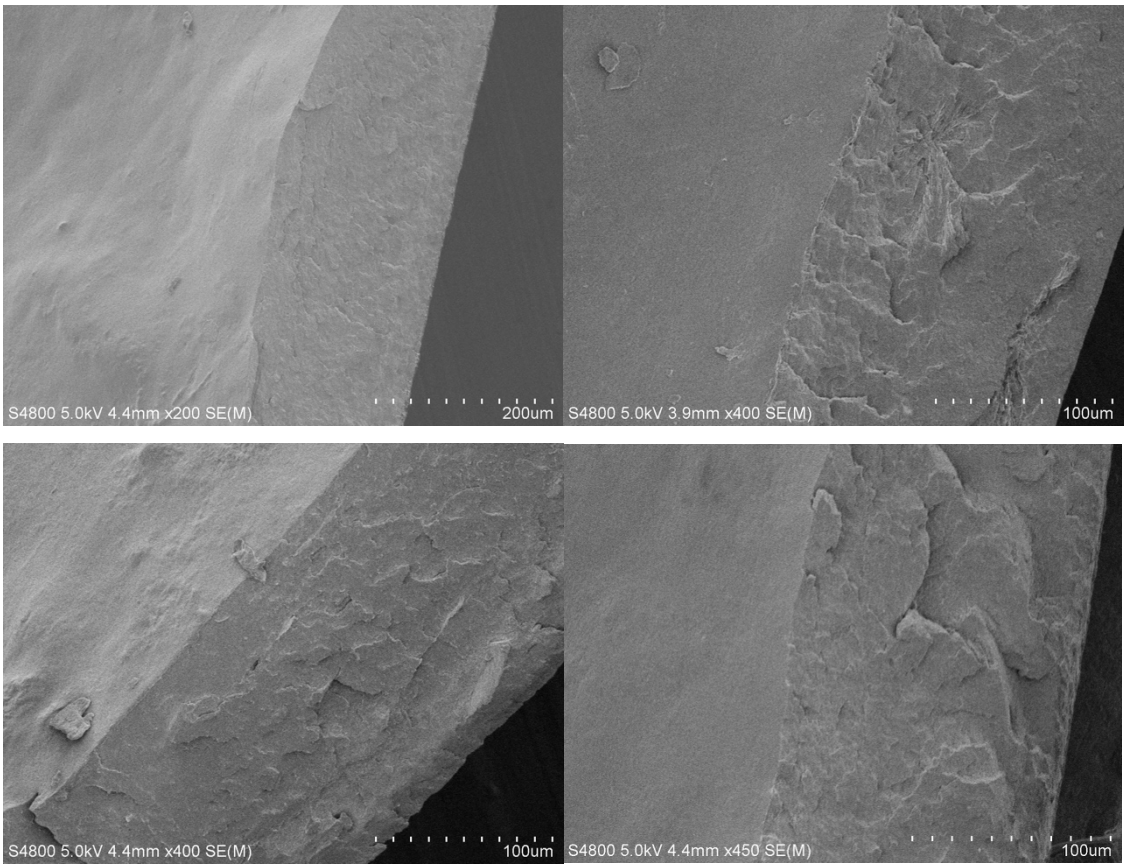


Figure 22: Cross section SEM image of CNF/PLA film with different CNF wt% and unmodified and acetylated fibrils. Top left: 2 wt% unmodified fibrils. Top right: 2 wt% acetylated fibrils. Bottom left: 5 wt% unmodified fibrils. Bottom right: 5 wt% acetylated.

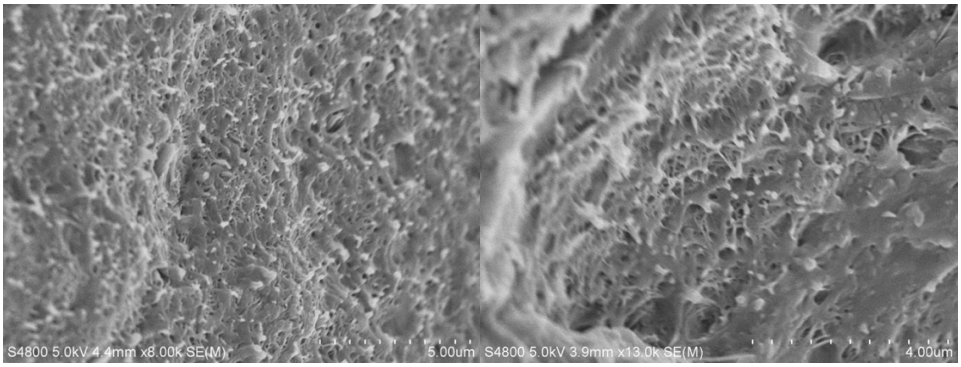


Figure 23: Prominent fibril network in PLA matrix. Left: 2 wt% unmodified CNF. Right: 5 wt% unmodified fibers.

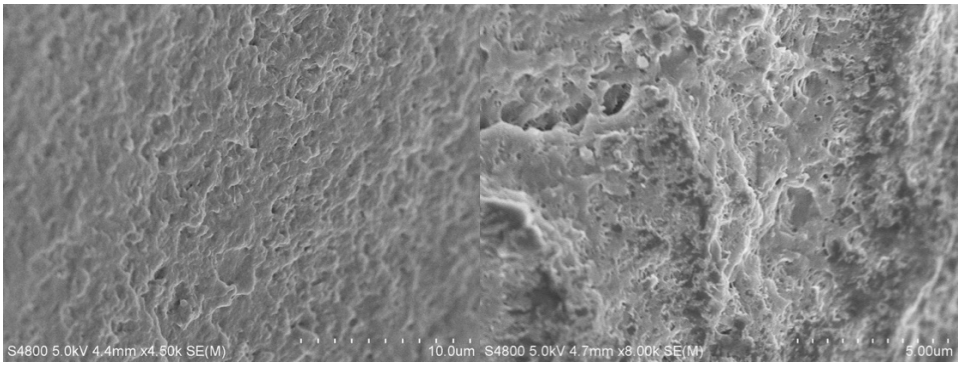


Figure 24: Cross-section of CNF/PLA film. Left: 2 wt% Acetylated CNF/PLA. Right: 5 wt% Acetylated CNF/PLA.

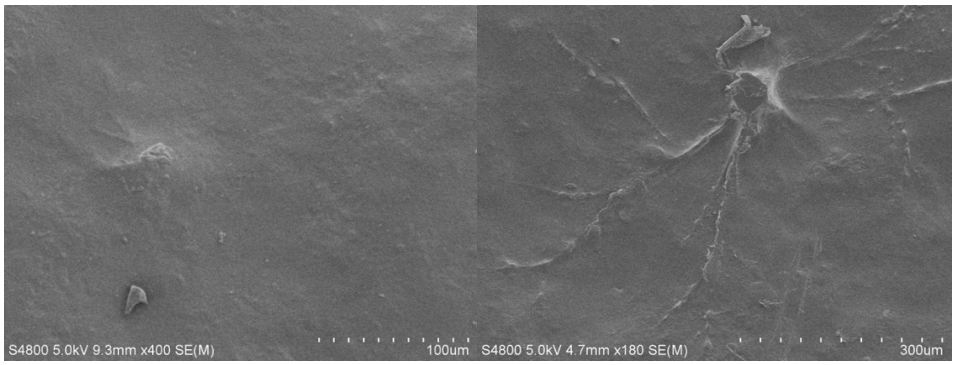


Figure 25: Surface defects. Left: 2 wt% unmodified CNF. Right: 5 wt% unmodified CNF

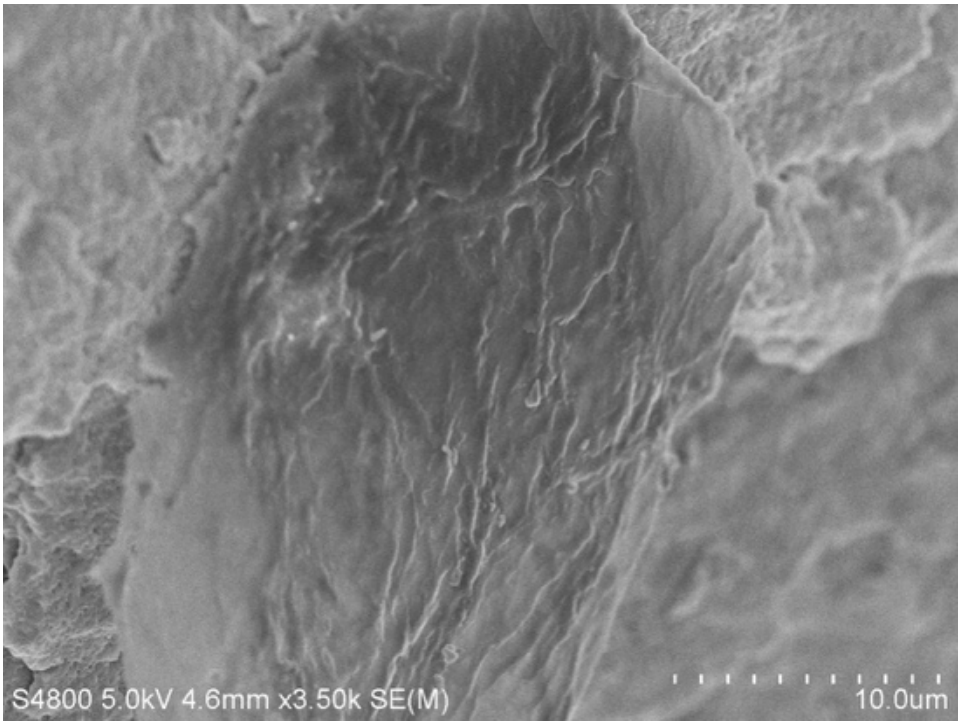


Figure 26: CNF/PLA with 5 wt% acetylated fibrils

4.1.4 Analysis of synthesis

PLA/CNF films were produced by transferring nanocellulose from a 1.7 wt% aqueous suspension to methanol, then to chloroform by a solvent exchange method. CNF and dissolved PLA were mixed before it was casted, and the solvent was evaporated. CNF containing films shown in Figure 20 had a slightly rough top surface and cloudy optical quality when compared to the almost completely transparent, smooth surface texture PLA film produced by the same solvent evaporation method. The method was successfully developed to produce consistent results for replication, but as expected it required large amounts of solvents which are lost when evaporated in the casting stage. The most complicated step was transferring the CNF between phases. The student wants to empathize the importance of a low CNF concentration and repeated S & C process described in section 3.3.3, especially between the aqueous phase and methanol. In the presence of too much water the methanol to chloroform S & C stage will due to the immiscibility of water and chloroform result in a drastic phase separation. It is possible to recover most of the CNF by transferring it back to methanol and further repeating the S & C process into

methanol. Examples of low quality films with aggregations and distinguishable regions caused by this phase separation are shown in Figure 21.

4.1.5 Analysis of tensile test results

Mechanical testing showed an obvious increase in strength and rigidity correlating with the CNF amount (Figure 22). Interestingly at 2 wt% loading the acetylated CNF caused a decrease in overall strength. Due to a too low quantity it is apparent that the fibrils did not form a network. The particular film also shows visual signs of uneven distribution (Figure 20). At 5 wt% loading it seems as the acetylated CNFs did in fact form a network which significantly improved the overall tensile strength. Contrary to Dufresne (2018) the ductility was drastically reduced in the films with unmodified fibrils most probably because of the lacking interface between components. The 5 wt% acetylated films performed well but exhibited a large variance in performance.

4.1.6 Analysis of SEM images

The rough surface was mainly caused by minor defects and variations in thickness and not accumulation of CNF as seen in Figure 24 and 27. The cross-sectional images of films with unmodified CNF seen in Figure 25 revealed fibrils sticking out of the matrix. Figure 25 also show holes which could have been caused by the removal of fibers when breaking the samples, or pores caused by trapped gas which would lower the density. Acetylated CNF/PLA films had smoother surfaces with less defects than the unmodified counterparts shown in Figure 24 and 26. The interface between fibril/polymer for the acetylated films (Figure 26) were significantly different in appearance compared to the unmodified fibrils (Figure 25), which could have been due to improved wetting. Figure 28 shows what could be an evenly distributed network of fibrils inside the polymer matrix. These images represent only small sections of the composite and do not guarantee that a percolated network has formed consistently throughout the samples.

5 CONCLUSION

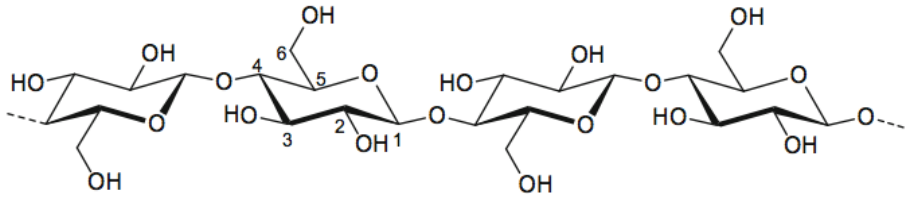
In this work nanocellulose was reviewed and CNF/PLA composites were produced, tested and visually analyzed to produce quantitative data for determining the overall potential of the composite material. Also, a surface treatment for increasing the hydrophobicity of CNF was clarified and implemented. The material testing showed an overall increase in tensile strength of CNF/PLA films with an exception of 2 wt% acetylated CNF/PLA film. It appears as low quantities of CNF significantly increased the tensile strength of PLA while reducing its ductility. The solvent casting method utilizes large amounts of solvents which are lost in the process and this is why other methods should be considered. As seen in SEM images there were no obvious aggregations in the fracture surfaces and films with acetylated CNF and the PLA did manage to wet the fibrils very well.

Nanocellulose could be produced at Arcada using some of the mentioned mechanical treatments and should be considered as it has immense potential and could be implemented in a wide variety of applications. For laboratory scale production there are also simpler methods available for example using a high-speed blender (Yano, 2003). The improved wetting of acetylated cellulose will possibly make re-dispersion of dried fibrils easier and allow proper dispersion using other compounding methods. It would be truly interesting to see the distribution of acetylated particles in a composite produced by extrusion.

As the general public encourages development of green design, the opportunity should be exploited (Dufresne, 2018). In Finland, commercialization of pilot scale projects has been supported by reorientation of large companies in the forest industry. Reorganization of universities has also brought together distant disciplines and sparked new initiatives which contribute to a more efficient bioeconomy (Sutinen, 2017). In packaging products trends show signs of shifting chemical grafting and modification to more high-end applications while large volume production will likely utilize aqueous suspensions and emulsions depending on application (Hubbe et al. 2017).

SAMMANDRAG

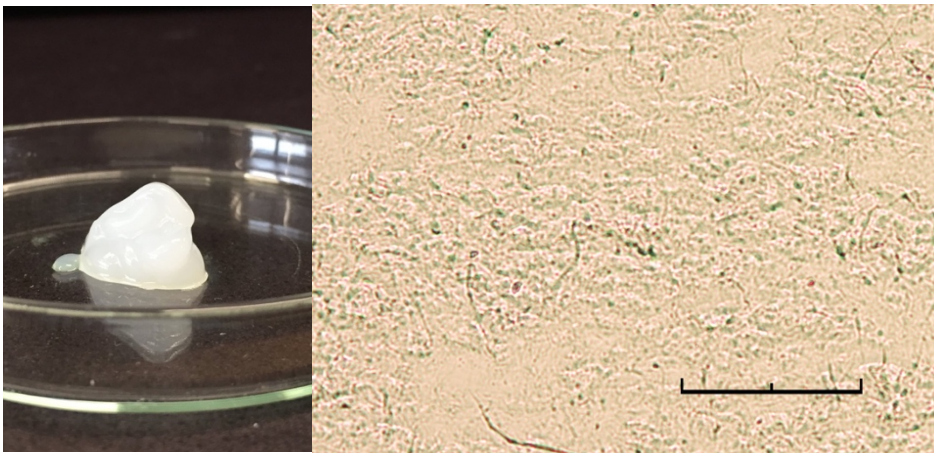
Vårdslös användning av oljebaserade plastprodukter har lett till föroreningar speciellt på avfallsplatser och vid kust- och sjöområden. En lösning till detta problem vore att ersätta en stor del av dessa med biopolymerer, plaster av biologiskt ursprung som bryts ner av naturliga processer till biomassa, gaser (CO_2, N_2), vatten samt organiska och oorganiska salter (Vijay et al, 2018). Biopolymererna har ofta i jämförelse med de vanligaste oljebaserade plasterna otillräckliga egenskaper, men kan användas i kombination med andra material i funktionsenliga kompositer (Thakur, 2018). Den alifatiska polyestern PLA anses vara en potentiell ersättare av många oljebaserade plaster på grund av stor produktionskapacitet och relativt låga priser (Ding et al. 2015). Den produceras av stärkelse från naturprodukter som majs, sockerbeta och kassava och är helt och hållet komposterbar (Fukushima et al. 2009, NatureWorks, 2012). Syftet med detta arbete var att studera produktionen av nanocellulosa samt användningen av små mängder nanocellulosafibriller (CNF) som fyllnadsmedel i bionedbrytbara plasten polylaktid (PLA). Världens mest allmänna biopolymer, cellulosa, finns i växter, alger, djur och mineraler (Heinze, 2016). I växter skapas polysackarider genom kondensationsreaktioner för olika ändamål. För lagring av energi i celler formas polysackarider i spiraler då däremot strukturella polysackarider skapar utsträckta formationer med begränsad rörlighet (Dufresne, 2018). Cellulosans monomerer D-glucopyranose ringarna består av fem kol och en syreatom i en stabil $^4C_1$-stolkonfiguration (Rojas, 2016). Ringarna med mycket reaktiva hydroxylgrupper i position C6, C2 och C3 är kopplade med β-1,4-glycosidbindningar vilka gör det möjligt för varannan ring att rotera 180 runt kedjans axel och skapa en lineär syndiotaktisk homopolymer.



Figur 1: Cellulosans polymerkedja (Rojas, 2016).

Kristalliteten av cellulosa är ojämnt fördelad och därför kan fibrerna delas upp i kristallina regioner med hög kristallinitet och amorfa regioner med lägre ordning (Klemm et

al. 2015). Den molekulära strukturen är den samma oberoende av källa, men i växter påverkas kristalliteten och de naturliga fibrernas sammansättning av botaniskt ursprung, klimat, jordmån och ålder (Ng et al. 2015). Denna mångsidiga resurs kunde utnyttjas bättre av länder med etablerad skogsindustri (RISE, 2015). Björk- och tallträdens stammar består av ca. 40% cellulosa och Finlands landyta är täckt till 86% av skog med en hållbar fällningsmängd på ca. 81 miljoner m^3 årligen (Finnish Natural Resources Institute, 2017, Sutinen, 2017). För att kompensera den sjunkande efterfrågan på pappersprodukter och som svar på den ökande efterfrågan av naturvänliga alternativ kunde företag satsa på produktion av produkter med ökat förädlingsvärde t.ex. Nanocellulosa (Sutinen, 2017). Även om cellulosan länge har använts inom pappersindustrin, identifierades dess elementära beståndsdelar nanocellulosafibriller (CNF) och nanocellulosakristaller (CNC) först på 1970-talet till följd av utvecklingen inom nanoteknik (Isogai et al, 2010). Sedan dess har andelen publikationer och forskning inom området ökat kraftigt. Denna ökning beror delvis på utveckling av relativt effektiva kemiska och mekaniska metoder för isolering av CNF och CNC som kan replikeras i laboratorier (Rissanen, 2017). Nanocellulosafibriller hanteras mestadels i vattensuspensioner med mycket låg koncentration p.g.a. exponentiellt ökande viskositet med mängden cellulosa (Kangas, 2013, Dufresne, 2018). De produceras med mekaniska, destruktiva uppifrån och ned-metoder och har då beroende av behandling och intensitet en varierande partikelstorlek (Rissanen, 2017) Den krävande processen är inte möjlig utan stora förluster i massa (Isogai et al. 2010). Till isolering av de elementära beståndsdelarna från naturliga växtfibrer används ofta en kombination av tre metoder. Traditionell högtryckshomogenisering som reducerar partikelstorleken till följd av stora tryckskillnader då suspensionen pressas genom en ventil. Microfluidizer teknik som pumpar en suspension igenom en z-formad korridor för att klyva fibrillerna vid kollision med väggarna. Högintensitetskvarnar med slipstenar som roterar upp till 1500 varm per minut är en effektiv metod för små produktioner (Kargarzadeh et al. 2017). CNF är vanligtvis 20-50 nm breda, 500-

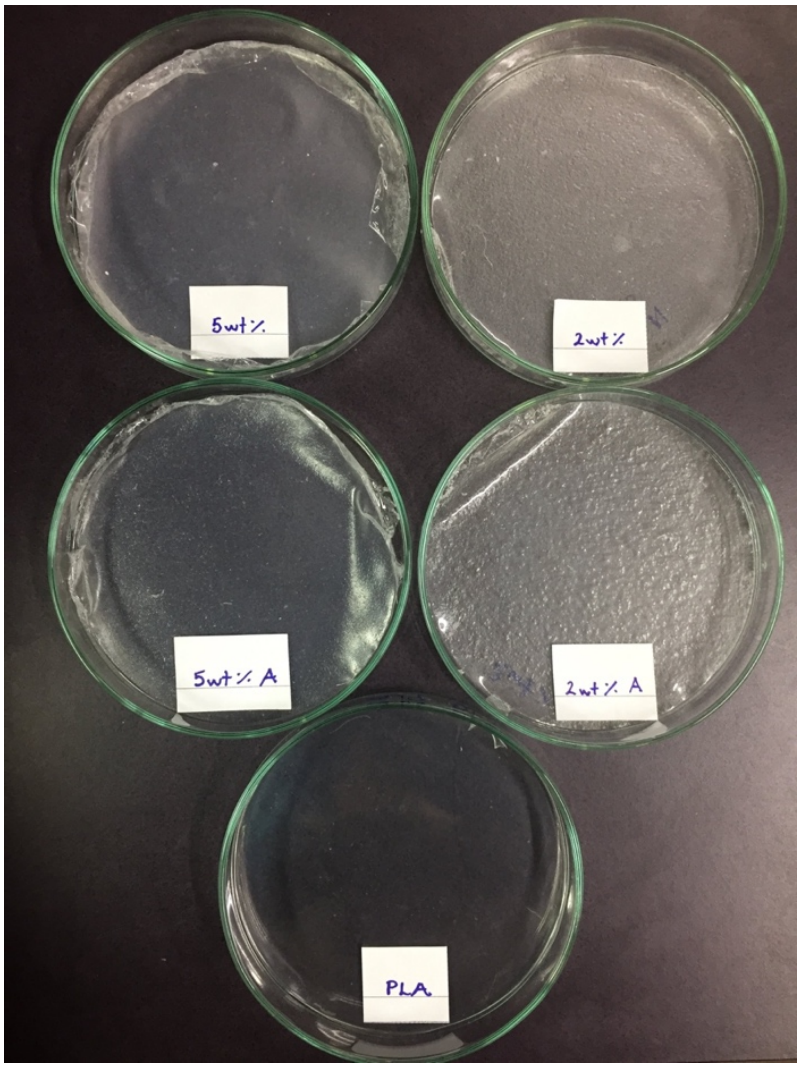


Figur 2: Vänster: CNF suspension. Höger: Torkad CNF under mikroskåp. 20x förstoring med en skala på 100µm

2000 nm långa med en specifik yta på 100-200m^2g^{-1} och kristallinitet mellan 60-70% (Kargarzadeh et al. 2017, Rissanen, 2017). Nanocellulosakristaller (CNC) isoleras genom selektiv syrahydrolys för att lösa upp de amorfa regionerna och slutliga produkten har oftast en kristallinitet under 80%. Kompositer är kombinationer av två eller fler material med olika faser och delar, ofta en kontinuerlig matris och en förstärkning dvs. partiklar eller fibrer (Vijay, 2018). Materialen är valda för att kombinera flera egenskaper och de slutliga egenskaperna påverkas av spridning, placering och gruppering av komponenter (Oksanen, 2016). För produktionen av nanocellulosakompositer är avlägsnande av vatten ofta ett problem. Ett välkänt fenomen inom pappersindustrin är cellulosans tendens att krympa och bilda irreversibla intermolekylära bindningar då den torkar (Zimmermann et al. 2016). Denna höga reaktivitet är också en delorsak till dess starka mekaniska egenskaper då fibrerna bildar täta nätverk (Dufresne, 2018). Sammanklumpning är ett framträdande problem i hydrofobiska matriser ifall den hydrofila cellulosan inte kommer åt att bilda ett nätverk. För att undvika detta steg kan cellulosan ytbehandlas för att minska polariteten, eller så kan kompositen produceras med andra metoder. Vattenlösliga polymerer undgår problemet helt och hållet då partiklarna direkt kan blandas in i en vattenlösning. I dessa material är också spridningen god och leder till en märkvärdig ökning i de mekaniska egenskaperna (Lee et al. 2014). Lösningsmedel kan också användas för att lösa upp en polymer, efter vilket en partikelsuspension i samma lösning blandas med. Då lösningsmedlet avdunstar har partiklarna p.g.a. Brownsk rörelse möjlighet att skapa ett nätverk. Enligt Dufresne (2018) är spridningen erhållen med denna produktionsmetod den bästa möjliga för alla polymera system. För att evalu-

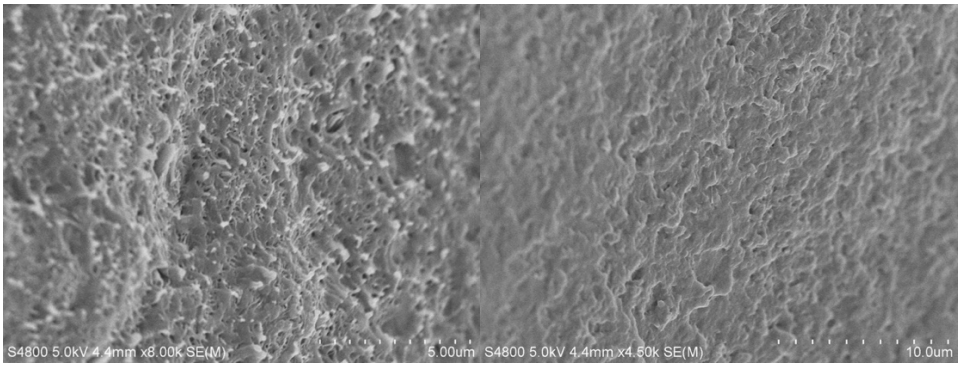
era egenskaperna av kompositer används flera olika metoder och maskiner. FTIR spektroskopi används för igenkänning av polymerer och additiv och DSC (Differential Scanning Calorimetry) för analys av övergångstemperaturer (Osswald, Menges, 2003, Naranjo et al. 2008). Dragprov görs för att samla kvantitativ information om olika material som används för forskning och utveckling (ASTM International, 2002). Optiska mikroskåp och elektronmikroskåp (SEM) kan användas för att visuellt analysera provbitar. I detta arbete undersöktes möjligheten för att skapa CNF/PLA kompositer med standard laboratorieutrustning och inverkan av CNF på blandningens mekaniska egenskaper i jämförelse med ren PLA. På grund av vissa begränsningar användes en avdunstningsmetod av Bulota et al. (2012) för att skapa filmer med lågt CNF innehåll. En CNF ytbehandling gjordes också för att göra ytan mer hydrofobisk och förbättra förenligheten med PLA matrisen. Ytbehandlingen gjordes genom att minska antalet reaktiva hydroxylgruppen på ytan genom esterifiering med hjälp av ättiksyraanhydrid och ättiksyra (Abdulkhani et al. 2014). Följande filmer producerades: 2% CNF/PLA, 2% acetylerad CNF/PLA, 5% CNF/PLA, 5% acetylerad CNF samt en film av ren PLA. Procenterna är givna i viktprocent. PLA hälldes i förseglingsbara Schott-flaskor var det löstes upp i kloroform. CNF vägdes upp i provrör i vilka de genomgick en homogenisering- och centrifugeringsprocess för att förflyttas till metanol och slutligen till kloroform. CNF/kloroformsuspensionen blandades med PLA/kloroformlösningen i Schott flaskorna före de hälldes i petriskålar behandlade med Chemlease 75 EZ formsläppmedel. Lösningsmedlet avdunstade över natten i ett välventilerat dragskåp efter vilket

de lösgjordes och konditionerades i fem dagar vid ungefärlig rumstemperatur och 50% luftfuktighet (Figur 2).



Figur 3: Producerade filmer. Översta raden från vänster: 5 viktprocent CNF/PLA, 2 viktprocent CNF/PLA. Mellersta raden från vänster; 5 viktprocent acetylerad CNF/PLA, 2 viktprocent CNF/PLA. Längst ned: PLA

Dragprov visade en ökad brottgräns med ökat CNF innehåll. Undantagsvis påvisade 2% acetylerad CNF/PLA film en lägre brottgräns än PLA. Detta är troligtvis på grund av den låga andelen då fibrillerna inte kan bilda ett nätverk som sträcker sig igenom hela kompositen.



Figur 4: Tvärsnitt av 2 viktprocent CNF/PLA film (Vänster) och 2 viktprocent acetylerad CNF/PLA film (höger) tagna med svepelektronmikroskop.

Närbilderna tagna med elektronmikroskop visar en tydlig skillnad på gränsytan mellan fibrillerna och matrisen för de omodifierade fibrillerna. I figur 3 sticker omodifierade fibillerna tydligt ur matrisen och har tätt intill kaviteter som kunde ha orsakats av instängd gas. En tydlig förbättring i vätbarhet uppmärksammades för acetylerade fibriller vilket bidrog till jämnare ytor och mindre brister. I detta arbete jämfördes olika förädlingsmetoder för nanocellulosa och produktionsmetoder för nanocellulosakompositer. CNF/PLA filmer producerades och analyserades med optiska metoder och destruktiva dragprov. Nanocellulosa är en användbar resurs som kunde användas vid Arcada i framtiden.

REFERENCES

Abdulkhani, A., Hosseinzadeh, J., Ashori, A., Dadashi, S. & Takzare, Z. 2014, "Preparation and characterization of modified cellulose nanofibers reinforced polylactic acid nanocomposite", *Polymer Testing,* , no. 35, pp. 73.

ASTM International 2002, *Standard Test Method for Tensile Properties of Thin Plastic Sheeting*, ASTM International, West Conshohocken, PA, United states.

Bulota, M., Kreitsmann, K., Hughes, M. & Paltakari, J. 2012, "Acetylated Microfibrillated Cellulose as Toughening agent in Poly(lactic acid)", *Journal of Applied Polymer Science,* , pp. 448.

Capadona, J.R., Ven Den Berg, O., Capadona, L.A., Schroeter, M., Rowan, S.J., Tyler, D.J. & Weder, C. 2007, "A versatile approach for the processing of polymer nanocomposites with self-allembled nanofibre templates.", *nnano,* vol. Nat Nanotechnol, no. 2, pp. 765.

Ding, W.D., Chu, R.K.M., Mark, L.H., Park, C.B. & Sain, M. 2015, "Non-isothermal crystallization behaviors of poly(lactic acid)/cellulose nanofiber composites in the presence of CO2", *Macromolecular Nanotechnology,* vol. 71, pp. 231.

Ding, W.D., Pervaiz, M. & Sain, M. 2018, "Cellulose-Enabled Polylactic Acid (PLA) Nanocomposites: Recent Developments and Emerging Trends" in *Functional Biopolymers*, eds. V.K. Thakur & M.K. Thakur, Springer International Publishin AG, Cham, Switzerland, pp. 183.

Diniz, J.M.B.F., Gil, M.H. & Castro, J.A.A.M. 2004, "Hornification - its origin and interpretation in wood pulps", *Wood Science Technology,* vol. 37, pp. 489.

Dufresne, A. 2018, *Nanocellulose : From Nature to High Performance Tailored Materials,* 2nd edn, Walter de Gryter Gmbh, Berlin/Boston.

Dufresne, A. 2013, "Nanocellulose: a new ageless bionanomaterial", *Materials Today,* vol. 16, no. 6, pp. 220.

European Commission 22/02/2017-last update*, Definition of a nanomaterial*. Available: http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm29/04/2018].

Fukushima, K., Abbate, C., Tabuani, D., Gennari, M. & Camino, G. 2009, "Biodegradation of poly(lactic acid) and its nanocomposites", *Polymer Degradation and Stability,* , no. 94, pp. 1646.

Granström, M. 2009, *Cellulose Derivatives: Synthesis, Properties and Applications*, University of Helsinki.

Heinze, T. 2016, "Cellulose: Structure and Properties", *Advances in Polymer Science,* vol. 271, pp. 1.

Henriksson, M., Henriksson, G., Berglund, L.A. & Lindström, T. 2007, "An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers", *European Polymer Journal,* vol. 43, pp. 3434.

Hubbe, M.A., Tayeb, P., Joyce, M., Tyagi, P., Kehoe, M., Dimic-Misic, K. & Pal, L. 2017, "Rheology of Nanocellulose-rich Aqueous Suspensions: A Review", *bioresources.com*.

Isogai, A., Saito, T. & Fukuzumi, H. 2011, "TEMPO-oxidized cellulose nanofibers", *Nanoscale,* vol. 3, pp. 71.

Jonoobi, M., Harun, J., Mathew, A.P., Hussein, M. & Oksman, K. 2010, "Preparation of cellulose nanofibers with hydrophobic surface characteristics", *Cellulose,* , no. 17, pp. 299.

Jonoobi, M., Mathew, A.P., Abdi, M.M., Makinejad, M.D. & Oksman, K. 2012, "A Comparison of Modified and Unmodified Cellulose Nanofiber Reinforced Polylactic Acid (PLA) Prepared by Twin Screw Extrusion", *Journal of Polymers and the Environment,* vol. 20, no. 4, pp. 991.

Kargarzadeh, H., Ionelovich, M., Ahmad, I., Thomas, S. & Dufresne, A. 2017, "Methods for Extraction of Nanocellulose from Various Sources" in *Handbook of Nanocellulose and Cellulose Nanocomposites*, eds. Kargarzadeh, Hanieh, Ahmad, Ishak, S. Thomas & A. Dufresne, First Edition edn, Wiley-VCH Verlag GmbH & Co. KGaA., , pp. 1.

Khalil, H.P.S.A., Bhat, A.H. & Yusra, A.F.I. 2012, "Green composites from sustainable cellulose nanofibrils: A review", *Carbohydrate Polymers,* , no. 87, pp. 963.

Khalil, H.P.S.A., Davoudpour, Y., Islam, M.N., Mustapha, A., Sudesh, K., Dungani, R. & Jawaid, M. 2014, "Production and modification of nanofibrillated cellulose using various mechanical processes: A review", *Carbohydrate Polymers,* , no. 99, pp. 649.

Klemm, P.D.D., Schmauder, P.D.H. & Heinze, P.D.T. 2015, "Cellulose" in *Biopolymers* Wiley, , pp. 275.

Law, K.L. 2017, "Plastics in the Marine Environment", *The Annual Review of Marine Science,* , pp. 205.

Lee, K., Aitomäki, Y., Berglund, L.A., Oksman, K. & Bismarck, A. 2014, "On the use of nanocellulose as reinforcement in polymer matrix composites", *Composites Science and Technology,* , no. 105, pp. 15.

Missoum, K., Belgacem, M.N. & Bras, J. 2013, "Nanofibrillated Cellulose Surface Modification: A Review", *Materials,* vol. 6, no. 5.

Murphy, C.A. & Collins, M.N. 2016, "Microcrystalline cellulose reinforced polylactic acid biocomposite filaments for 3D printing", *Polymer Composites,* vol. 39, no. 4, pp. 1311.

Naranjo, A., del Pilar Noriega E., Maria, Osswald, T.A., Roldan-Alxate, A. & Sierra, J.D. 2008, *Plastics Testing and Characterization,* Carl Hanser Verlag, Munich.

Natural Resources Institute Finland 2017, 27.06.2017-last update*, Total roundwood removals and drain* [Homepage of Natural Resources Institute Finland], [Online]. Available: http://stat.luke.fi/en/roundwood-removals-and-drain [2018, 02/14].

NatureWorks. 2012, "NatureWorks Ingeo Polylactide: Past, Present and Future", *Biopolymers & Biocomposites Workshop*Iowa State University, Minnetonka, MN, 14 Augut 2012.

Ng, H., Sin, L.T., Tee, T., Bee, S., Hui, D., Low, C. & Rahman, A.R. 2015, "Extraction of cellulose nanocrystals from plant sources for application as reinforcing agent in polymers", *Composites Part B,* , no. 75, pp. 176.

Oksman, K., Aitomäki, Y., Aji, M.P., Siqueira, G., Zhou, Q., Butylina, S., Tanpichai, S., Zhou, X. & Hooshmand, S. 2015, "Review of the recent developments in cellulose nanocomposite processing", *Composites,* , no. Part A 83, pp. 2.

Osswald, T.A. & Menges, G. 2003, "Mechanical Behavior of Polymers" in *Materials Science of Polymers for Engineers*, 2nd Edition edn, Hanser Publishers, Munich, pp. 420.

Österberg, M., Vartiainen, J., Lucenius, J., Hippi, U., Seppälä, J., Serimaa, R. & Laine, J. 2013, "A Fast Method to Produce Strong NFC Films as a Platform for Barrier and Functional Materials", *Applied Materials & Interfaces,* .

Räisänen, T. & Athanassiadis, D. 2013, "Basic chemical composition of the biomass components of Pine, Spruce and Birch", .

Research Institutes of Sweden AB 2015, *Roadmap 2015 to 2025: Materials from nanocellulose*, RISE, www.ri.se.

Rissanen, V. 2016, *PROCESS OPTIMIZATION OF CELLULOSE FIBRIL PRODUCTION - THE EFFECT OF PROCESS MEDIUM COMPOSITION ON ENERGY EFFICIENCY AND PRODUCT QUALITY*, Aalto University.

Sabu, T., Kuruvilla, J., S. K. Malhotra, Koichi, G. & M. S. Sreekala 2013, "Biocomposites" in *Polymer Composites* John Wiley & Sons, Incorporated, , pp. 362.

Scheirs, J. 2000, "Sampling an Sample Preparation" in *Compositional and Failure Analysis of Polymers; A Practical Approach* John Wiley & Sons, Ltd, England, pp. 30.

Sutinen, R. 2017, *Wood-Based Bioeconomy Solving Global Challenges*, Ministry of Economic Affairs and Employment; Enterprise and Innovation Department.

Swain, S.K., Pattanayak, A.J. & Sahoo, A.P. 2018, "Funtional Biopolymer Composites" in *Functional Biopolymers*, eds. V.K. Thakur & M.K. Thakur, Springer, .

Tardy, B.L., Yokota, S., Ago, M., Xiang, W., Kondo, T., Bordes, R. & Rojas, O.J. 2017, "Nanocellulose–surfactant interactions", *Current Opinion in Colloid & Interface Science,* , no. 29, pp. 57.

Testometric Co. Ltd. 2018, *, Testometric M350-5 CT* [Homepage of Testometric Co. Ltd], [Online]. Available: https://www.testometric.co.uk/5kn/ [2018, 04/25].

Thakur, V.K. & Thakur, M.K. 2018, *Functional Biopolymers,* Springer, Cham, Switzerland.

Tingaut, P., Zimmermann, T. & Lopez-Suevos, F. 2010, "Synthesis and Characterization of Bionanocomposites with Tunable Properties from Poly(lactic acid) and Acetylated Microfibrillated Cellulose", *Biomacromolecules,* vol. 11, pp. 454.

Yano, H. 2003, *, Current situation of Nanocellulose in Japan* [Homepage of Tappi], [Online]. Available: http://www.tappi.org/content/events/11NANOSTDS/paper/yano.pdf [2018, .

Zhu, Y., Romain, C. & Williams, C.K. 2016, "Sustainable polymers from renewable resources", *Nature,* , no. 540, pp. 354.

Zimmermann, M., Borsoi, C., Lavoratti, A., Zanini, M., Zattera, A.J. & Santana, R.M. 2016, "Drying techniques applied to cellulose nanofibers", *Reinforced plastics & composites,* vol. 35, no. 8, pp. 682.